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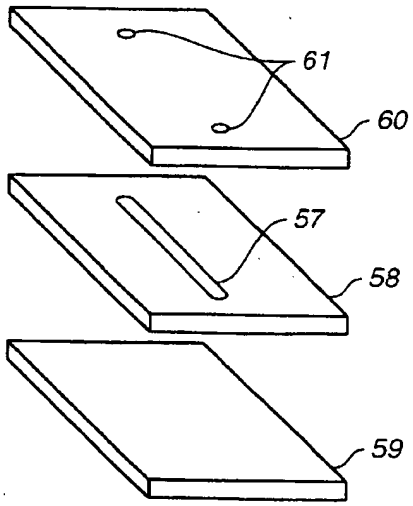

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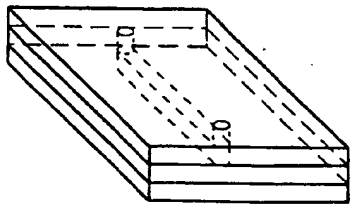

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(57) Abstract: The present invention provides modular microfluidic devices and systems, as well as methods for their manufacture. A microfluidic device is provided comprising first and second substrates (59, 60), and at least one stencil (58) sandwiched between the first and second substrates so as to define one or more sealed microstructures therebetween. The stencil is adhered to at least one of the first and second substrates by an adhesive (44). In a preferred embodiment, there is a plurality of sandwiched stencils. Also, the first and second substrates are preferably substantially planar. These microfluidic devices can be rapidly prototyped with low tool-up cost, and can be easily assembled to form three-dimensional structures having complex microfluidic system geometries.

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MODULAR MICROFLUIDIC DEVICES COMPRISING SANDWICHED STENCILS

This application claims the benefit of co-pending U.S. Provisional Application Serial No. 60/157,565, filed October 4, 1999.

FIELD OF THE INVENTION

The present invention relates generally to modular microfluidic devices and components that can be combined together to form microfluidic devices. More specifically, the present invention relates to modular microfluidic devices comprising layered substrates and sandwiched stencils, and processes for their manufacture.

BACKGROUND OF THE INVENTION

There has been a growing interest in the manufacture and use of microfluidic devices for the acquisition of chemical and biological information. In particular, microfluidic devices allow, for example, complicated biochemical reactions to be carried out using very small volumes of liquid. These miniaturized devices increase the response time of the reactions, minimize sample volume, and lower reagent cost, among other benefits.

Traditionally, microfluidic devices have been constructed in a planar fashion using techniques borrowed from the silicon fabrication industry. Representative devices are described, for example, in some early work by Manz *et al.* (Trends in Anal. Chem. (1990) **10(5)**: 144-149; Advances in Chromatography (1993) **33**: 1-66). In these publications, microfluidic devices are constructed by using photolithography to define channels on silicon or glass substrates and etching techniques to remove material from the substrate to form the channels. A cover plate is bonded to the top of this device to provide closure. Miniature pumps and valves can also be constructed to be integral with (e.g., within) such devices.

More recently, methods have been developed that allow microfluidic devices to be constructed from plastic, silicone or other polymeric materials. In one such method, a negative mold is first constructed, and plastic or silicone is then poured into or over the mold. The mold can be constructed using a silicon wafer (see, e.g., Duffy *et al.*, Anal. Chem. (1998) **70**: 4974-4984; McCormick *et al.*, Anal. Chem. (1997) **69**: 2626 –2630), or by building a traditional injection molding cavity for plastic devices. Some molding facilities have developed techniques to construct extremely small molds. Components constructed using a LIGA technique have

been developed at the Karolsruhe Nuclear Research Center in Germany (see, e.g., Schomburg *et al.*, Journal of Micromechanical Microengineering (1994) **4**: 186-191), and commercialized by MicroParts (Dortmund, Germany). Jenoptik (Jena, Germany) also uses LIGA and a hot-embossing technique. Imprinting methods in polymethylmethacrylate (PMMA) have also been described (see, e.g., Martynova *et al.*, Anal. Chem. (1997) **69**: 4783-4789). However, these techniques do not lend themselves to rapid prototyping and manufacturing flexibility. Additionally, these techniques are limited to planar (i.e., two-dimensional, or 2-D) microfluidic structures. Moreover, the tool-up costs for these techniques are quite high and can be cost-prohibitive.

Gonzalez *et al.* have described a modular approach to microfluidics (Sensors and Actuators B (1998) **49**: 40-45). However, the microfluidic devices described by Gonzalez *et al.* are not truly modular since they are limited to interconnect systems for silicon wafers.

In view of the foregoing, there exists a need for low-cost, prototypable modular microfluidic devices that can be readily combined to construct more complex microfluidic systems. In addition, there is a need for modular, three-dimensional microfluidic devices, which can include various componentry (e.g., valves, filters, etc.).

SUMMARY OF THE INVENTION

The present invention addresses the foregoing needs and provides additional advantages over existing microfluidics technology. The modular approach of the microfluidic devices of the present invention, and the flexibility and low-cost manufacturing processes involved, permit the construction of microfluidic systems comprising "generic" device components which may be easily and effectively assembled or combined to meet a wide variety of design considerations and requirements. The modular design obviates the need for the design and manufacture of costly custom microfluidic systems.

One object of the present invention is to provide an inexpensive and robust modular microfluidic device. An additional object is to provide a microfluidic device that is rapidly prototyped with minimal tool-up costs. The manufacturing cost of the microfluidic devices of the present invention is relatively low, at both high and low production volumes.

Another object of the present invention is to provide a modular system of microfluidic components that can be combined in various configurations to construct a microfluidic device. In this manner, prototyping and manufacturing can be accomplished in a very rapid manner, since a complete set of generic "building block" components can be constructed in bulk. These

components and devices can then be combined in various ways to construct desired microfluidic systems.

Yet another object of the present invention is to provide an inexpensive means of manufacturing positive or negative molds for the construction of microfluidic replicates.

An additional object of the present invention is to provide "built-in" (i.e., integrated) electronic components within the microfluidic devices. Specifically, for example, electrodes can be placed within channels and chambers of the microfluidic devices. These electrodes can be used for electrokinetic flow, electrophoresis, electrochemical detection, impedance measurements and temperature sensing, among other functions.

Another object of the present invention is to provide a microfluidic device comprising valves for the controlled handling and manipulation of fluids.

Another object of the present invention is to provide a microfluidic device comprising a microstructure capable of filtering a small volume of fluid, especially a fluid comprising biomolecules such as nucleic acids or proteins.

An additional object of the present invention is to provide a microfluidic device that is chemically compatible with or can accommodate the use of a vast array of liquid reagents or solutions including, but not limited to, organic solvents such as acetonitrile.

These and other objects are provided by the present invention. In a preferred embodiment, a microfluidic device is provided comprising first and second substrates, and at least one stencil disposed (e.g., sandwiched) between the first and second substrates so as to define one or more sealed microstructures therebetween. The stencil is adhered to at least one of the first and second substrates by an adhesive. In a preferred embodiment, there is a plurality of sandwiched stencils. Preferably, the first and second substrates are substantially planar, and have surfaces complementary with each other so as to better seal microstructures therebetween. The first and second substrates preferably are made from Mylar®, FR-4, polyester, glass, acrylic, polycarbonate or fiberglass.

The adhesive is preferably a rubber-based adhesive, an acrylic-based adhesive or a gum-based adhesive. In a preferred embodiment, the stencil is self-adhesive. In a most preferred embodiment, the stencil comprises an adhesive tape, which can be single-sided (i.e., have adhesive on one side) or double-sided (i.e., have adhesive on both sides). Any adhesive tape may be used, including especially commercially available adhesive tapes. Examples of types of adhesive tape include, but are not limited to, pressure-sensitive tapes, temperature-activated (e.g., heat activated) tapes, chemically-activated (e.g., two-part epoxy) tapes and

optically-activated (e.g., UV-activated) tapes. Preferably, the adhesive tape comprises a backing material selected from the group consisting of Mylar®, nylon and polyester, to support the adhesive. In an alternate embodiment, the stencil and at least one of the first and second substrates are ultrasonically welded together.

The stencil can be made from polymers, papers, fabrics and foils, among other materials. Preferably, the stencil comprises a polymer selected from the group consisting of Mylar®, polyesters, polyimides, vinyls, acrylics, polycarbonates, Teflon®, Kapton®, polyurethanes, polyethylenes, polypropylenes, polyvinylidene fluorides, polytetrafluoroethylenes, nylons, polyethersulfones, acetal copolymers polyesterimides, polysulfones, polyphenylsulfones, ABS, polyvinylidene fluorides, polyphenylene oxides, and derivatives thereof. In one preferred embodiment, the stencil comprises a fluorinated polymer, which are known to be chemically-resistant. The stencil may be made from an elastomeric material, such as, for example, rubber, viton or silicone. Preferably, stencils for use in the present invention are die-cut by, for example, an automated rotary die-cutting machine.

The microfluidic device of the present invention preferably further comprises a sealant coat on at least a portion of one or more of the stencil, the first substrate and the second substrate. The sealant coat can help adhere the substrates and the stencil(s) together, and help seal the microstructure(s) defined therebetween. The sealant coat preferably comprises a silicone material. Alternatively, the sealant coat can comprise one or more of Teflon®, Avatrel®, Liquin®, fluorocarbons, fluorothermoplastics, polyvinylidene fluorides, acrylics, waxes, epoxies, solders, polymers, paints, oils and varnishes. The sealant coat can be applied by a number of different methods, including spin-deposition, spraying and dipping.

The microfluidic device preferably includes one or more microstructures comprising a channel or chamber. In certain applications, the microstructure is at least partially filled with a filling material, such as a filter material. The filter material may comprise a wide variety of materials capable of specific and non-specific filtering of various size parameters. Any of various chemical, biological and size-exclusion filter materials may be used. In certain embodiments, the filter material is selected from the group consisting of polycarbonates, acrylics, acrylamides, polyurethanes, polyethylenes, polypropylenes, polyvinylidene fluorides, polytetrafluoroethylenes, naphion, nylons and polyethersulfones. The filter material may also be selected from the group consisting of agarose, alginate, starch and carrageenan. Preferably, the filter material is Sephadex®, Sephacil® or hydroxyapatite. In a preferred embodiment, the filling material is applied by silk screening, which can reduce the manufacturing time and cost.

The filling material can also be applied using lithography. Preferably, the filling material is applied using pick-and-place techniques, which are well known in the semiconductor manufacturing industries.

In certain embodiments, the microfluidic device includes at least one substrate having one or more apertures, which can be in fluid communication with one or more substrates. The microfluidic device may also further comprise one or more valves of various designs. Several valve configurations and componentry are described below. The microfluidic device can be used to divide a liquid sample into a plurality of sample. In one embodiment, such splitting of samples is accomplished by using a microstructure comprising one or more forked channels, each preferably having one or more constrictions to control fluid flow therethrough.

The microfluidic device may further comprise at least one electrode. The electrode can be used for detecting or measuring an electrical property of a fluid. Alternatively, the electrode is for promoting electrophoretic or electrokinetic flow.

The microfluidic device may also be used in conjunction with external optical spectroscopy, interrogation and detection. Thus, in certain preferred embodiments, at least a portion of at least one of the substrates in the microfluidic device is adapted to permit transmission of an optical signal (i.e., is sufficiently optically transparent).

The microfluidic device of the present invention may be multi-layered to form a three-dimensional device or system. Therefore, in certain embodiments, the microfluidic device may further comprise one or more additional substrates sealingly engaged thereto. The additional substrate(s) are preferably layered or stacked so that microstructures of the various layers are sufficiently aligned as to be functional for the desired application. One or more of these additional substrates can comprise a circuit board having on a surface thereof a microstructure. Circuit board substrates useful in the construction of microfluidic devices are described in co-pending United States Patent Application Serial No. _____ (Attorney Docket No. _____), the disclosure of which is incorporated herein by reference. In addition, a microfluidic system comprising a plurality of microfluidic devices may be constructed, wherein at least two of the microfluidic devices are configured to enable fluid communication with each other. The microfluidic system can be prepared by layering two or more microfluidic devices to form a three-dimensional microfluidic system.

The present invention also provides a method for producing a microfluidic device. The method comprises the steps of: (a) providing a first substrate; (b) layering on the first substrate one or more panels, each comprising an array of stencils; and (c) layering on the one or more

panels a second substrate so as to define a plurality of microstructures therebetween. Preferably, at least one of the first and second substrates has one or more apertures. Also, it is preferred that at least one of the panels is aligned with at least one of the first and second substrat s so that the apertures are in fluid communication with the microstructures. Such alignment is preferably provided by peg-and-hole alignment. The present invention also provides in certain embodiments microfluidic devices prepared according to the foregoing method.

The present invention also provides a mold prepared using at least a portion of a stencil as a form for defining the mold. The mold preferably is made from a silicone material. A microfluidic device comprising a microstructure can be prepared using such a mold.

Definitions

The term "microfluidic" as used herein is to be understood to refer to microstructures wherein one or more of the dimensions is less than 500 microns, as well as to components, devices and systems comprising such microstructures. The microfluidic devices of the present invention can be planar (i.e., approximately two-dimensional, or 2-D) or three-dimensional (3-D). Additionally, such devices can be constructed using any of the materials described herein, as well as combinations of such materials, and similar or equivalent materials.

The term "microstructure" as used herein refers to microfluidic structures disposed on one or more substrates used to assemble the microfluidic devices of the present invention. The term encompasses any of a variety of structures (including, but not limited to, channels and chambers) that are capable of supporting a fluid (i.e., microstructures through or into which fluid(s) are capable of being passed, stored or directed). The microstructure boundaries are defined by the outline of the cut-away portion(s) of the sandwiched stencils.

The term "sealed" as used herein refers to a microstructure having a sufficiently low unintended leakage rate and/or volume under given flow, fluid identity and pressure conditions. The term also encompasses microstructures that have one or more apertures therein through which fluid is intended to pass.

The term "adhesive" as used herein refers to any chemical having adhesive properties so as to be effective to adhere the various stencil and/or substrate layers of a microfluidic device of the present invention together to define a sealed microstructure therebetween.

The term "channel" or "chamber" as used herein is not intended to be restricted to elongated configurations where the transverse or longitudinal dimension greatly exceeds the

thickness, depth or cross-sectional dimension. Rather, such terms are meant to comprise cavities or tunnels of any desired shape or configuration into or through which liquids may be directed or passed. Such a fluid cavity may, for example, comprise a flow-through channel where fluid is to be continually passed or, alternatively, a chamber for holding a specified, discrete amount of fluid. "Channels" and "chambers" may be filled or may contain internal structures comprising, for example, valves, filters, and similar or equivalent components and materials.

The term "stencil" as used herein refers to a material that is preferably substantially planar from which one or more variously shaped and oriented portions are cut or removed. The outlines of the cut or removed portions comprise the lateral boundaries of microstructures that are formed upon sandwiching stencil(s) between substrates.

The microfluidic devices described here are "generic" in that they are modular and can be easily reconfigured into or adapted to any design. These devices are capable of being used with a variety of pumping and valving mechanisms, including pressure, peristaltic pumping, electrokinetic flow, electrophoresis, vacuum and the like. In addition, the microfluidic devices of the present invention are capable of being used in collaboration with optical detection (e.g., fluorescence, phosphorescence, luminescence, absorbance and colorimetry), electrochemical detection, and any of various suitable detection methods. Suitable detection methods will depend on the geometry and composition of the device. The choice of an appropriate detection method for a given application is within the purview of the skilled artisan.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the construction of a microfluidic device comprising a stencil sandwiched between two substrates. Figure 1A is an exploded perspective view showing the individual components of the device, and Figure 1B is a three-dimensional perspective view of the assembled device.

Figure 2 provides cross-sectional views (A-D) showing a stencil being coated with a sealant coat. The adjacent substrate (cover plate) can optionally also be coated with a sealant coat, as in Figure 2D.

Figure 3 is a cross-sectional view showing a microfluidic device where one sealing coat material is used to coat the stencil and underlying substrate, and a second sealant coat material is used to help seal the substrates together.

Figure 4 shows the construction of a three-dimensional microfluidic device comprising

stencils. Figure 4A is an exploded perspective view showing the individual components, and Figure 4B is a three-dimensional perspective view of the assembled device.

Figure 5 shows the construction of another three-dimensional device comprising a stencil. Figure 5A is an exploded perspective view showing the individual components, and Figure 5B is a three-dimensional perspective view of the assembled device.

Figure 6 illustrates the use of silk screening technology to fill or coat specific areas (e.g., filter chambers) of a microfluidic device. Figure 6A shows the individual components, and Figure 6B shows an alignment procedure for silk screening a panel of devices.

Figure 7 is an exploded perspective view of a microfluidic device that has an integrated (i.e., "built-in") valving mechanism.

Figure 8 is a top view of a microfluidic device comprising forked channels capable of splitting a sample into four approximately equal parts.

Figure 9 illustrates the components of a microfluidic device that has an integrated (i.e., "built-in") valving mechanism.

Figure 10 is an exploded view showing an alignment technique using peg-and-hole alignment technology to ensure proper alignment of various layers and componentry of microfluidic devices.

Figures 11A and 11B are photomicrographs of a microfluidic device comprising a sandwiched stencil, showing fluid passing therethrough at two stages of operation.

Figure 12A shows the components of a microfluidic device constructed using 18 stencils. Figures 12B and 12C are photomicrographs of such a device with acetonitrile passing through it at two stages of operation.

Figure 13A shows the components of a microfluidic device that is constructed using 9 stencils. Figures 13B and 13C are photomicrographs of such a device with water passing through it at two stages of operation.

Figures 14A-14C are photomicrographs of water passing through a microfluidic device comprising a chamber filled with silica gel.

Figure 15 is a photomicrograph of a microfluidic device constructed using a stencil comprising a thermal (i.e., temperature-activated) tape.

Figure 16 shows the construction of a microfluidic device comprising both circuit board-type substrates and stencils. Figure 16A illustrates at left an exploded perspective view and at right an assembled perspective view. Figure 16B is a photomicrograph of a device as shown in Figure 16A with fluid passing therethrough.

Figures 17A and 17B are photomicrographs of a microfluidic device comprising forked channels capable of splitting or dividing a fluid sample, at two stages of operation.

Figures 18A-18D are photomicrographs of a microfluidic device having a built-in filter and a built-in valve, with fluid passing therethrough at four stages of operation.

Figures 19A and 19B show the use of a stencil as a mold for generating microfluidic replicates. Figure 19C is a photomicrograph of a silicone microfluidic replicate made using such a mold, with fluid passing therethrough.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS OF THE INVENTION

Referring to Figure 1, a microfluidic device is constructed by sandwiching a stencil between two substrates. Referring to Figure 1A, a channel 57 is constructed by sandwiching a stencil 58 between two substrates, here represented by a bottom substrate 59 and a top substrate 60. In this embodiment, both the inlet and outlet apertures 61 are positioned in the top substrate 60. The assembled device is shown in Figure 1B. The inlet and outlet apertures can either lead to the outside of the device or to an adjacent stencil and/or substrate layer. In another embodiment (not shown), stencil layers are stacked directly on each other, rather than being sandwiched between substrates.

Microstructures (e.g., channels and chambers) can be formed in the stencil either before or after being placed onto a supporting substrate. In a preferred embodiment, the stencil is shaped prior to placement on a substrate, by cutting or removing a portion of the stencil material of the appropriate shape and orientation. The stencil can be cut, for example, using a die-cutter, which is preferably automated. Alternatively, the cutting may be performed using a laser cutter. In a preferred embodiment, the stencil is automatically cut using a die cutter or a laser cutter that is controlled by a computer. In another preferred embodiment, the cuts are made using a rotary cutter or printer press, or any high throughput auto-aligning equipment. Such equipment is often referred to as converters.

In a preferred embodiment, the stencil comprises single-sided or double-sided adhesive tape. A portion of the tape (of the desired shape and dimensions) can be cut and removed to form, for example, a channel or chamber. The tape stencil can then be placed on a supporting substrate or between substrates. In one embodiment, stencil layers are stacked on each other. The thickness or height of the channels can be varied by simply varying the thickness of the stencil (e.g., the tape carrier and the adhesive thereon).

Various types of tape are useful in the above embodiment. The type of adhesive can be

varied to accommodate the application, as can the underlying carrier's thickness and composition. Suitable tapes for use in the present invention can have various methods of curing or activation, including pressure-sensitive tapes, temperature-activated tapes, chemically-activated tapes, optically-activated tapes, among other types of tapes. Various adhesives are useful, including, for example, rubber-based adhesives, acrylic-based adhesives, and gum-based adhesives. The materials used to carry the adhesive are also numerous. Examples of suitable tape carrier materials include Mylar®, polyester and nylon. The thickness of the carrier can be varied.

In a preferred embodiment, a probe is used to define the channels and chambers of the stencil. In one embodiment, the probe is a cutting device mounted to, for example, a computer-controlled plotter. The probe selectively removes shapes from a material to form a stencil d fining the lateral boundaries of microstructures (e.g., channels and chambers). In one mbodiment, a heat probe is used to selectively melt or anneal heat-activated adhesive to form microstructures. In another embodiment, ultrasonic welding is used to create microstructures in layeres stencils. For example, channels can be defined in two stencil layers. These layers can be "melted" together using ultrasonic welding.

In a preferred embodiment, the material that forms the stencil is applied onto the substrate in only certain desired areas using silk screening. The material is then "cured" to form the channels and/or chambers. Examples include the use of an activatable or curable polymer as the stencil material. Another example is the use of paint or ink as the material. One example is the use of a Thick Medium heat-set acrylic from Genesis Artist Colors (Indianapolis, IN). In another embodiment, the entire surface of one of the substrates is coated with the stencil material. The stencil is then cured in areas where it is to remain and the rest of the material can be removed. In this embodiment, a curable epoxy material may be used. In a more preferred embodiment, the epoxy is a UV-curable epoxy. Alternatively, a two-part epoxy can be used, where the first part is patterned into place and the entire device is then soaked in the second part that only adheres the stencil material in certain areas.

The chemical nature of the stencil material and, thus, the microstructure's chemistry, can be "tuned" for particular applications. The stencil material can be hydrophilic, hydrophobic or ionic in nature. The stencil material can be flexible. In various preferred embodiments, the stencil material is selected from the group consisting of vinyl, filter material, paper or fabric, foil, and foam or foam sheets. In other preferred embodiments, the stencil material is formed from a polymeric material. Suitable polymers include, but are not limited, to polycarbonate, acrylic,

polyurethane, polyethylene, including high-density polyethylene (HDPE) and ultra-high molecular weight polyethylene (UHMW), polypropylene (PP), polyvinylidene fluoride (PVDF), polytetrafluoroethylene (PTFE), nylon, polyethersulfone (PES), acetal copolymers, polyesterimides, polysulfones, polyphenylsulfones, ABS, polyvinylidene fluoride, polyphenylene oxide, and derivatives thereof. In an especially preferred embodiment, the polymer is a fluorinated polymer, since fluorinated polymers often have superior resistance to aggressive solvents such as organic solvents.

In a preferred embodiment, the stencil material is a flexible or elastomeric material, such as silicone, viton, or rubber, so as to enable valving and pumping mechanisms. Pressure or mechanical force can be applied to the flexible layer to cause the material to bend and block a channel located above or below it.

In a preferred embodiment, the sealant coat can serve to both coat and seal a microstructure. Referring to Figure 2, at least a portion of the surface of the stencil 33 and/or substrate 30 can be coated with a layer of a sealant coat material 38. A cover plate substrate 39 (which is preferably substantially planar) can be layered upon the stencil 33 to "cap" or complete the microstructure 35 defined between substrates 30 and 39. In Figure 2C, the cover plate substrate 39 is not coated. In Figure 2D, the cover plate substrate 39 is coated with a sealant coat material 41, which can be the same as or different than the sealant coat material 38. Referring to Figure 3, dabs of epoxy 44 are added to help adhere cover plate substrate 39, substrate 30 and stencil 33 together. The epoxy 44 can be added either before or after the sealant coat material 38 has been cured. In another preferred embodiment, the layers of the device can additionally be held together mechanically. Gaskets may, for example, be used in order to help seal the microstructures.

Numerous suitable sealant coat materials having various desired properties can be used. The sealant coat material can be chemical and/or biological in nature, and can be hydrophobic or hydrophilic, depending on the application. Solids, liquids, gels and powders, or combinations thereof, can be used. Materials capable of carrying a surface charge can be used, as can neutral species. Specific examples of coating materials suitable for use in the present invention include Teflon®, Liquin®, Avatrel®, silicone, silicone mixtures, epoxies (including solder masks), glue, liquid polymers, polymeric dispersions, plastic, liquid acrylic, paint, metals, oils, waxes, photoresist, varnish, solder and glass.

In a preferred embodiment, the sealant coat material is a polymer, such as, for example, polyethylene glycol and cyanoacrylate. In other preferred embodiments, the coating material is

biological in nature. Advantageously, in various applications, the biological coating material can be used to either promote or prevent adherence of materials. In certain embodiments, a biological coating material (e.g., a ligand) that specifically binds to certain biological materials is preferred. Examples of biological coating materials include proteins, antibodies, lipids, cells, tissues, nucleic acids and peptides. More specific examples include avidin, streptavidin, poly-lysine, and enzymes. In certain embodiments, the coating materials are used to selectively bind materials that are present in the samples.

In another preferred embodiment, the sealant coat material is a fluorinated polymer. Fluorinated polymers have excellent resistance to various solvents and chemicals, including organic solvents. Examples include Teflon®, Avatrel®, polyvinylidene fluoride (PVDF), THV Fluorothermoplastic (Dyneon, St Paul MN), Hostaflon TF 5035 (Dyneon), among others.

The various sealant coat material(s) can be deposited using a number of techniques. In a preferred embodiment, the sealant coat material(s) are spin-deposited onto a given substrate and/or stencil using a spinner or rotator. Specifically, an appropriate amount of a sealant coat material is placed on a substrate or stencil and the entire substrate or stencil is spun to produce a generally uniform sealant coat layer. In a preferred embodiment, the spin rate is between about 10 rotations per minute (rpm) and about 100,000 rpm. More preferably, the spin rate is about 500-20,000 rpm and, most preferably, is about 1,000-20,000 rpm. In order to make the coating thicker, multiple spin-deposition cycles can be used.

Alternatively, the sealant coat material can be deposited by spraying the sealant coat material onto a surface. For example, the sealant coat material can be ultrasonically sprayed through a nozzle or other orifice. In one embodiment, colloidal dispersions of the sealant coat material are prepared, the concentration being adjusted so that when sprayed onto a surface, a layer of desired thickness results. In another embodiment, the sealant coat material is sprayed directly onto a surface. In yet another embodiment, the sealant coat material is dissolved in an appropriate solvent and then sprayed onto the surface; when the solvent evaporates, the sealant coat material is left behind to form a coating layer. The sealant coat material can, alternatively, be applied by dipping a substrate and/or stencil into a volume of the sealant coat material. A single dip may produce a coating of a certain thickness; in order to make the coating thicker, multiple dips may be applied. Alternatively, the sealant coat material can be brushed onto a surface. As with the spraying techniques described above, the sealant coat material can be deposited directly as a colloidal dispersion, or as a material dissolved in a solvent. In another preferred embodiment, the sealant coat material is stamped onto a surface.

In a preferred embodiment, the sealant coat material is patterned (e.g., by silk screening techniques) onto a surface. In this embodiment, the sealant coat material can be used to coat only certain selected areas of the surface as defined by the silk screening mask. In another preferred embodiment, photoresist patterning can be used to achieve liftoff or etch patterning. The photoresist can then be removed to leave a coating only on certain areas of the surface. This procedure can be repeated as desired or necessary using different photoresist patterns and coating materials.

In alternate embodiments, a variety of thin film deposition techniques can be used to deposit sealant coat materials. Such techniques include, but are not limited to, thermal evaporation, e-beam evaporation, sputtering, chemical vapor deposition, and laser deposition. These and other thin film deposition techniques are well known in the art. In addition, plating techniques can be used to deposit sealant coat materials. Such plating techniques include, but are not limited to, electroplating of metallic materials and chemical plating.

The thickness of the sealant coat may be important in certain embodiments. Preferably, the thickness of the coating is sufficient to chemically protect the underlying surface and/or to adhere or seal an adjacent substrate and/or stencil. A potential problem of too thick a coating is the obstruction or blockage of microstructures, which can impede or prevent fluid flow therein.

Where the sealant coat material does not solely serve an adherence function, thinner coatings can be used. In fact, a molecular layer (or monolayer) may be preferable in certain instances. In a preferred embodiment, the sealant coat is a self-assembled monolayer of alkane thiols, which is particularly amenable to deposition on metal surfaces such as gold. Other similar thiols can be used. In another preferred embodiment, silanization reactions can be used to coat the substrates. Silanization is known to minimize adherence of certain biological materials such as nucleic acids and peptides. In yet another preferred embodiment, the microstructures are coated with a lipid bilayer or multilayer. In certain embodiments, these molecular monolayers are terminated with a biological molecule that is used to bind a molecule in the solution. Examples include nucleic acid-terminated alkane thiols and protein-terminated silanes.

In certain embodiments, a secondary mechanism may be used to help seal substrates and/or stencils together. In certain embodiments, these layers are held together mechanically. Examples include using nuts and bolts, tight-fitting pegs and holes, epoxy, BLU-TEK®, or an external clamp. Alternatively, pressure or vacuum can be used to accomplish this mechanical adhesion or sealing.

It is sometimes necessary to adjust the viscosity of the sealant coat material prior to the coating step. In order to obtain a desired viscosity, some of the sealant coat materials may need to be diluted or thinned with other solvents or chemicals. Alternatively, the sealant coat materials can be heated prior to their deposition to alter their viscosity. Appropriate viscosity adjustments will be apparent to those skilled in the art.

Substrates and stencils to be coated are preferably cleaned prior to the coating and adhesion steps. Examples of cleaning techniques include soaking, sonicating, rinsing and plasma cleaning. Examples of cleaning materials include soap, surfactants, detergents, organic solvents and Freon®.

In another preferred embodiment, flexible sealant coat materials can be used on certain layers of the device in order to enable valving and pumping mechanisms. A preferred flexible sealant coat material is silicone rubber. Pressure or mechanical force can be applied to the flexible layer to cause the material to bend and block a channel located above or below it.

Three-dimensional structures can be formed using stencils defining channels and/or chambers. Referring to Figure 4, a microfluidic device comprising a filter is constructed. A T-channel 62 is cut in a stencil 64 comprising double-sided tape, which is adhered to a top plate 66 having two entry ports 68. A stencil supporting layer 70 is adhered to the bottom of stencil 64. Supporting substrate layer 70 has an aperture 72 that leads to adjacent stencil layer 74 that is double-sided tape from which has been cut and removed a filter channel 76 and a filter chamber 78. The filter chamber 78 can be filled with any of a variety of suitable filter materials, depending on the given application (see examples below). A bottom substrate 80 is adhered to stencil layer 74 having the filtering componentry. An outlet aperture 81 is formed in the bottom substrate 80. In operation, a sample is injected into one of the inlet apertures 68 and a reagent is injected into the other of the inlet apertures 68. The sample and the reagent mix at the junction of the T-channel and are passed to the adjacent layers below. The filter material in filter chamber 78 captures unwanted material(s), and the purified material passes through the outlet aperture. The assembled device is shown in Figure 4B.

An alternate 3-D microfluidic device design is shown in Figure 5. In this embodiment, the top three layers are identical to those in Figure 4. However, instead of constructing a channel for the filter and filling it with an appropriate filter material, layer 84 is itself a filter material. The bottom layer 80 having outlet aperture 81 is the same as in Figure 4. The assembled device is shown in Figure 5B.

Methods of forming apertures include, but are not limited to, mechanical drilling, laser

drilling, chemical etching, plasma etching and hole punching. Alternatively, components of the device can be constructed from injection molded parts that have integrated or built-in inlet/outlet apertures. Other techniques known in the art for through-hole formation can be employed.

In certain embodiments, the sealant coat materials can be chemically bonded to the underlying substrate and to the next layer. Alternatively, non-covalent chemical interactions can b used to hold the substrates together. The stencil material can be melted onto the underlying substrate or adhered using an adhesive or some other mechanism, such as heating. In other mbodiments, the stencil can be mechanically pressed onto the underlying or adjacent substrate.

A circuit board substrate having on a surface thereof a microstructure may comprise one or more layers of a microfluidic device. The use of circuit board-type substrates, and other substrates having metal laminates, in constructing microfluidic devices is the subject of co-pending United States Patent Application Serial No. _____ (Attorney Docket No. _____), the disclosure of which is incorporated herein by reference.

In a preferred embodiment, a microstructure can be filled with any of a variety of filling materials, including reagents or catalysts. These filling materials, in certain embodiments, can be used to perform useful chemical and/or biological reactions. In a preferred embodiment, the filling materials are filters, which are useful for separating and/or purifying materials. These filters can be chemical or biological filters, or size-exclusion filters. These filters may bind unwanted material or, alternatively, may bind the material of interest so that it may be eluted off later. The filling materials can be hydrophobic or hydrophilic in nature, and can be charged or neutral. The filling material may be porous with various pore sizes. In a preferred embodiment, the filling material used to fill a channel or chamber is polymeric. Examples include, but are not limited to, polycarbonate, acrylic, polyurethane, high-density polyethylene (HDPE), ultra-high molecular weight polyethylene (UHMW), polypropylene (PP), polyvinylidene fluoride (PVDF), polytetrafluoroethylene (PTFE), naphion, nylon, and polyethersulfone (PES). In a preferred embodiment, the material used to fill the channel is a carbohydrate, such as agarose, alginate, starch, or carrageenan. The polymer may also be an electro-active polymer. In a preferred embodiment, the filling material is silica gel. In another preferred embodiment, the filling material is Sephadex® or Sephacil®. In another preferred embodiment, the material used to fill the channel is acrylamide or agarose. In another preferred embodiment, the material used to fill the channel is hydroxyapatite.

In a preferred embodiment, the filling material used to fill the channel and/or chamber is

a biological material. Examples include, but are not limited to, binding proteins, antibodies, antigens, lectin, enzymes, lipids, and any molecules that may interact specifically or non-specifically with one or more of the species in the fluid.

In a preferred embodiment, the filling material is composed of a powder, such as charcoal or porous beads. In another preferred embodiment, the filling material is a reagent that is to be activated during the use of the device. Two examples are soluble reagents and catalysts.

In a preferred embodiment, the filling material is a paper filter. This filter may be a commercially available material that is chemically modified to perform a specific function, such as binding a material or filtering a variety of materials.

In a preferred embodiment, the materials placed in the microstructures perform useful biological or chemical functions. Examples include solid buffer materials that can be used to buffer a sample once injected. Other materials include lysis buffer for lysing cells and solid reagents. Catalysts can also be placed within the devices.

In a preferred embodiment, the filling material is composed of a single component that is already formed prior to being placed into a microstructure. Alternatively, the material can be formed from multiple components that can be separately placed into a channel; once in the channel, the materials can react to form the final filling material. Such curing can be accomplished in a variety of ways, and can be spontaneous or catalyzed by some other mechanism such as light, heat, a catalyst, solvent, drying, etc.

In one embodiment, the filling material is placed into the microstructures during the manufacturing process. In this manner, high-throughput techniques can be used to fill the channels. In one embodiment, high-throughput pick-and-place equipment, like that used in the electronics industry, is used to place the filter materials.

In one embodiment, the filling material is patterned into the microstructures by, for example, silk screening the material into the channels, or by using lithography, or by mechanically placing the material. Referring to Figure 6A, an "empty" microfluidic device 90 having two filter chambers 91 and 92 is shown. In order to place filter material in the filter chambers 91 and 92, two silk screens 93 and 94 are created. The screen and stencil are formed using materials that are compatible with the filter to be screened. A variety of screen materials and stencils can be used. One of the screens is aligned, for example, above the device and the first filter material 95 from screen 93 is screened onto the device. The second filter material 96 can subsequently be screened onto the device from screen 94 in a similar

fashion to create the "completed device 98. The viscosity and thickness of the material to be screened is preferably adjusted to properly fill the filter chamber or other filter area. Additionally, the amount of screened-on material should be adjusted so that the chamber fills properly.

In a preferred embodiment, an entire panel of devices can be coated simultaneously. Referring to Figure 6B, stencils are constructed on a panel 111. A preferred panel size is approximately 18" by 24"; however, other panel sizes may be used. Fiducial marks 114 are placed on the panels for visual or optical alignment. Holes placed in the stencil are used to align the stencil on the various machines used during the device manufacturing process. Silk screens 112 and 113 comprising filter material are aligned with the devices on panel 111. A single alignment allows all of the filter chambers (see 115 and 116 denoting the two types of filter chambers on the panel 111) to be filled simultaneously. Finally, substrates and/or additional stencils may be added to complete a microfluidic system.

In a preferred embodiment, one or more of the layers of a microfluidic device of the present invention can be used as a valve. Referring to Figure 7, a multi-layer purification device is constructed. An inlet aperture 100 and an outlet aperture 101 are constructed in a top substrate layer 102. In one embodiment, this substrate layer can be a thin piece of acrylic, glass or polycarbonate. A stencil forms the next layer below. In this stencil, a filter chamber 104 is constructed and filled with an appropriate filter material (not shown) by, for example, silk-screening. Referring again to Figure 7, a T-shaped feature is formed, where one arm of the T is a chamber 105 and the other arm is a channel 103. A hole 106 is located at the distal end of the chamber 105. Channel 103 is in communication with outlet aperture 101 in the top substrate layer 102. The bottom substrate layer 110 of the stack comprises a filter material that allows air to flow but that becomes blocked when liquid comes into contact with it. Examples of such filter materials include X-7744, a 7 μm pore size T3 sheet from Porex Technologies (Fairburn, GA) and Goretex®-type materials. In operation, a sample is injected into the inlet aperture 100. The sample flows through the filter material in chamber 104 and into "wide" chamber 105 and "large" hole 106 until sufficient volume has been injected to fill that chamber and hole. Then, the fluid flows into "narrow" channel 103 and out through outlet aperture 101. Any number of mechanisms can be used to force the fluid to preferentially flow into the larger chamber 105 first. Capillary forces may be taken advantage of. Alternatively, the channels may be coated with materials that induce preferential flow.

An alternative valving mechanism can be used where pressure and/or vacuum is applied to inlet and outlet apertures from an external source. Rate of fluid flow can be controlled by

varying the external pressure or flow of the input mechanism. Examples include the use of a syringe or peristaltic pump. Alternatively, lateral microconstrictions can be defined in a channel to limit the rate of flow. Alternatively, vertical constrictions can be added inside a channel. These constrictions can also be used to facilitate mixing. Alternatively, rate of flow in a network of channels can be limited or controlled by forcing the liquid through a channel or portion thereof coated with a compound more hydrophobic than the rest of the network.

In a preferred embodiment, a microfluidic device is used to concentrate samples. The device is constructed so that the volume of the wide channel/chamber and the large hole is about 2-100,000 times larger than the remaining filter chamber and channel volume. A large sample can be injected and washed many times. Then, a very small volume of eluent can be added to remove the sample that had been adhered to the filter material in filter chamber 104.

In another preferred embodiment, valves are constructed by altering the shapes of the channels themselves. Referring to Figure 8B, a device is created having multiple channel splits or forks 120. After each split, the channels are constricted (see 122 in Figure 8B) so that local capillary forces are increased in that region. Further splits can be constructed after the constricted region. Referring to Figure 8B, as a sample enters a split or fork, the fluid typically will prefentailly pass down one of the two channels of the split. However, once the fluid reaches the constriction area, the other of the two channels becomes filled because the capillary forces in this region are much less than in the constricted section. Once both channels are filled, the fluid passes the constriction regions to the next channels, and so on. Using this approach, samples can be accurately distributed into approximately equal portions. A three-dimensional device using this design should permit samples to be split into many partitions.

In another preferred embodiment, a valve is constructed by altering the outlet apertures for each channel. Referring to Figure 9C, a microfluidic device is constructed that has a large primary channel 125 with a smaller branched channel 126. The primary channel 125 terminates in channel 127, which is narrower than channels 125 and 126. A cover plate substrate 128 with inlet and outlet apertures positioned at the end of each of the aforementioned channel forms the top substrate layer. When fluid enters the main inlet aperture 129, the fluid flows first down the primary channel 125. When the fluid reaches the junction of channel 127, the capillary forces are sufficient to force the fluid down channel 126.

The surface chemistry of the various channels may be altered in order to achieve the same goal. In one embodiment, the end of a large channel is coated with a hydrophobic material, while the rest of the channels are hydrophilic. When water enters the large channel, it

first passes down the large channel. When it reaches the area where the channel has been derivatized with hydrophobic terminal groups or coatings, surface tension forces the remainder of the water down the smaller, hydrophilic channels. Similar techniques can be used with organic solvents.

With multi-layered microfluidic devices, alignment of the layers is a consideration. Therefore, preferably, automated techniques will be used for this alignment. Additionally, the construction of these devices will be done in parallel so that a single alignment can be performed for the construction of a multitude of devices. In a preferred embodiment, peg-and-hole techniques are used to align the layers. Referring to Figure 10, a manifold 130 is constructed having dowels 132 of specified diameter. A panel 134 of stencils is placed on the manifold 130; alignment is accomplished by aligning holes 135 with the dowels 132. An adjacent layer 136 is placed atop panel 134; similarly, alignment is accomplished by aligning holes 137 with the dowels 132. Such manifolds can also be used to accommodate silk screens.

In another preferred embodiment, edge alignment is used. The edges of the devices are aligned, which automatically aligns the channels and chambers if the devices are cut to specified dimensions. Alignment can be accomplished mechanically, optically, magnetically, or otherwise.

In a preferred embodiment, a panel of devices is simultaneously aligned and processed. The completed panel of structures may then be cut or sectioned into individual devices. In a preferred embodiment, the component layers are scored into device sections prior to assembly.

In another preferred embodiment, the layers of the device are brought in close proximity, realigned, and then pressed together. In this and other embodiments it may be important to control the humidity and temperature of the environment surrounding the layers. Often, the coating materials and adhesives cause local static interactions between the layers, making alignment difficult. In an additional embodiment, the layers can be aligned under water, to minimize the static. In another embodiment, a small amount of soap is added to the water to prevent immediate adhesion if a slight misalignment occurs. In another embodiment, the temperature of the local environment surrounding the layers is controlled in order to aid in alignment.

In another embodiment, a press is constructed that has alignment blocks on both the top plate and bottom plate. One layer of the device is placed in the bottom alignment jig, and the next layer is placed in the top alignment jig. The automated press then brings the devices together for adhesion. When the press is expanded, the two layers stay on the bottom block.

The next layer can be applied in a similar manner.

A converter or die-cut printing machine can be used for constructing and layering stencils. For example, the materials that form each stencil layer may be loaded in roll format onto the machine. Mechanical die punches are made for each layer. The materials are rolled out, punched, and laminated together in an automated fashion. Companies such as Acutek (Los Angeles, CA) provide services using converters. In a similar manner, a rolling system could be used to fill channels and chambers. The roll of devices can be pulled across an alignment block as described above, and filter material can be added. Again, alignment may be accomplished using a peg-and-hole alignment block. However, other alignment techniques may be used, including optical alignment and precision placement.

In a preferred embodiment of this invention, electrodes are placed in the channels and chambers to perform, for example, detection and/or activation functions. Examples include lectrophoresis, electrokinetic flow, electrochemical detection, impedance detection, capacitive detection, heating and measuring current or voltage. In another embodiment, interesting structures are created to induce other phenomena. For example, current can be passed through metal lines disposed in a microstructure to induce heating within the microstructure. Thermocouples can be constructed within the microstructure using the metal lines to detect thermal changes. Calorimetry can be performed in this manner. In addition, a magnetic field can be induced in a similar manner. This magnetic field can be used to detect certain phenomena or induce flow using magnetic particles.

In another preferred embodiment, the stencils are not used as the fluidic devices themselves, but rather they (or a portion thereof) are used as forms to define a positive or negative mold. Various molding materials can be used, such as moldable polycarbonate or various silicones (see, e.g., Duffy *et al.*). Microfluidic devices can be prepared comprising microstructures formed using such molds.

The following Examples describe certain aspects of several preferred embodiments of the present invention and are not intended to be limiting in any manner. Rather, the scope of the present invention is defined by the claims appended hereto. Other materials and configurations, not specifically disclosed herein, are also contemplated. Such alternate embodiments will be apparent to one of ordinary skill in the art in view of the present disclosure.

EXAMPLES

Exampl 1

A three-dimensional microfluidic device was constructed using double-sided adhesive tap as a stencil. Specifically, a stencil was prepared by cutting a channel from a piece of #444 double-sided transparent polyester film tape (3M, St Paul, MN). This tape stencil was mounted onto a 1/16" thick acrylic sheet having a 0.04" diameter inlet aperture aligned with one end of the stencil channel. A 2 mil thick Mylar® sheet was then layered on the tape stencil; the Mylar® substrate had a 0.04" diameter aperture aligned with the other end of the stencil channel. Another tape stencil comprising a channel was then positioned on the Mylar® substrate. Finally, an acrylic cover substrate was placed on the tape; this acrylic substrate had a 0.04" diameter outlet aperture at the opposite end of the stencil channel from the Mylar® hole. Photomicrographs of this microfluidic device are provided in Figure 11 with water passing therethrough. Figures 11A and 11B show water on the first layer and the upper channel layer, respectively.

Example 2

A three-dimensional microfluidic device was constructed as follows. Modular components were constructed by preparing stencils comprising channels by cutting a self-adhesive laminating sheet tape (Avery Dennison, LS10P, 73603) using a computer-controlled plotter modified to have a cutting blade. Seven of these modules were designed so that they could be reconfigured (using simple orientation changes) to construct various microfluidic devices. In this example, two different microfluidic devices were constructed using these modules. In both cases, the first stencil was placed on a 1/16" thick polycarbonate sheet substrate having a drilled 33 mil hole as an inlet aperture. In one device, the remaining stencils were layered in the order shown in Figure 12A (i.e., 1,2,3,4,4,4,5,3,6,7,5,4,3,6,3,5,7,1), so that fluid could pass from one layer to the next at specific locations designated by the round features. The final substrate was a piece of Avery Dennison LS10P tape having an outlet aperture. In this 17-layer microfluidic device, fluid enters and exits from the same direction. Figures 12B and 12C are photomicrographs of the device with colored acetonitrile passing therethrough at two stages of operation. An alternative device was constructed using five of the same modules, but by altering their layering order and orientation to be (1,2,3,4,4,4,4,7,1), as shown in Figure 13A. Figures 13B and 13C are photomicrographs of the device with colored water passing therethrough at two stages of operation.

Exampl 3

A microfluidic device comprising a filter chamber was constructed. A stencil was prepared by cutting a channel out of a 1 mil thick vinyl sheet having adhesive on one side. This stencil was placed on a 1/16" acrylic sheet having an inlet aperture aligned with either end of the filter. A silica gel slurry was made by mixing 10 parts of 50 mM NaCl (aq) with 1 part silica gel having a 40 μm average particle diameter (JT Baker Chemical Co., Phillipsburg NJ). The filter chamber was filled with the slurry by screening it in place. A similarly-shaped stencil was prepared by cutting a piece of #444 double-sided tape (3M). The tape stencil was generally aligned with and placed on the vinyl stencil, and an acrylic cover substrate was then placed on the tape stencil. Colored fluid was passed through the device (and the filter chamber) and no leakage was observed.

Example 4

A microfluidic device comprising a filter chamber was constructed. A stencil was prepared by cutting a channel from a 1 mil thick vinyl sheet having adhesive on one side. This stencil was placed on a 1/16" acrylic sheet having an inlet aperture and an outlet aperture aligned with either end of the filter chamber. A sheet of filter paper (Whatman Product # 1070) (Whatman Limited, England) was cut and placed onto the stencil in the filter chamber area. Two outlet apertures were disposed on the opposite side of the filter with a built-in valving mechanism as described above. A 25 μm pore size Porex filter was used as the valving mechanism. A sample is passed across the filter. The remaining sample and a number of wash cycles are passed through the filter, which then pass into an ante-chamber located beyond the filter chamber. Once sufficient wash cycles have been employed, a plug of elution buffer is passed across the filter. The valve unit is then activated, and the elution plug passes into the ante-chamber for analysis or further manipulation. This device is useful to purify and concentrate nucleic acids, particularly where the filter material is hydroxyapatite. Figure 14 provides three photomicrographs (A-C) of the device at various stages of operation.

Example 5

A microfluidic device was constructed as follows. A stencil was prepared by cutting a channel from a layer of heat-sealable nylon tape (Product #4220; Bemis Associates, Inc., Shirley, MA), which is 3 mils thick and anneals at 108°C. This stencil was sandwiched between a 2 mil thick Mylar® substrate and a 1/16" thick polycarbonate substrate having two inlet apertures. An arbor press was fitted with heated aluminum plates. The plates were pre-heated to ~ 108°C. The device was placed in the press and compressed, at temperature, for 2-3

seconds to seal the device. Figure 15 is a photomicrograph of the assembled device with fluid passing through it.

Example 6

Referring to Figure 7, a microfluidic device designed for biochemical (e.g., protein) purification applications is shown. Two apertures 100 and 101 (each 40 mil diameter), representing inlet and outlet apertures, respectively, are drilled in a 1/8" thick polycarbonate substrate 102. Stencils are constructed by cutting channels out of a piece of self-adhesive laminating sheet tape (Avery Dennison, LS10P, 73603) using a computer-controlled plotter modified to have a cutting blade (see Figures 12 and 13). The filter chamber 104 is then filled with Bio-Gel® HTP hydroxyapatite (Biorad, Hercules, CA). An 80 mil diameter hole 106 is drilled in a 1/8" thick polycarbonate substrate 108, and a sheet of PTFE (25 micron pore size) from Porex Technologies (Fairburn, GA) is used as the bottom substrate 110.

A 100 nl volume of protein solution in 10 mM phosphate buffer is added to the inlet aperture 100. The protein binds to the hydroxyapatite filter 104, while the other biomass does not. The volume of the chamber 105 and the 80 mil hole 106 is adjusted to accommodate the sample volume (100 nl) plus 4 equivalent washes with buffer (400 nl). Once this amount of fluid has washed the filter 104, 100 nl of higher salt concentration buffer is then injected. The molarity of the salt in the elution buffer will differ for different types of proteins; typically, 400 mM phosphate buffer is sufficient. This solution elutes the protein off the filter 104. The eluent is then collected at the outlet aperture 101. The bottom substrate 110 acts as a passive capillary valve by pumping the eluent through the outlet aperture 101 by capillary forces.

Example 7

A three-dimensional microfluidic device was constructed comprising channels formed using both circuit board substrates and adhesive tape stencils. Referring to Figure 16A, which shows at left an exploded perspective view of the individual components of the device, channels 150 and 151 were formed on a circuit board substrate 160 and coated with a silicone sealant coat. Inlet aperture 200 and outlet aperture 201 were formed in the circuit board substrate within channels 150 and 151, respectively. An acrylic cover plate substrate 202, having two apertures 204 and 205, was attached to the top of the coated circuit board channels 150 and 151. A roughly horseshoe-shaped channel 206 was constructed in a stencil 208 cut from #444 double sided tape (3M), and the stencil was aligned and adhered to the acrylic substrate 202. Finally, an acrylic substrate 210 was placed on the other side of the tape stencil 208. The assembled microfluidic device is shown at right in Figure 16A.

Fluid is injected at inlet aperture 200 and travels down the first circuit board channel 150. The fluid than travels through aperture 204, and then to and through the tape stencil channel 206. The fluid eventually passes through aperture 205 into the second circuit board channel 151 and exits through outlet aperture 201. Figure 16B is a photomicrograph of a device according to Figure 16A with fluid passing therethrough.

A device such as this can be used to perform electrophoretic or electrokinetic separation. Electrodes can be provided, for example, at the inlet and outlet apertures to apply the appropriate voltages. Since the optically transparent tape stencil layer 208 extends further than the optically opaque circuit board, analysis of the fluid contained in stencil channel 206 is possible using a variety of optical techniques.

Example 8

A sample-splitting microfluidic device comprising forked channels was constructed. A stencil as shown in Figure 8B was constructed by cutting the outlined area out of a one-sided self-adhesive laminating sheet tape (Avery Dennison, LS10P, 73603) using a computer-controlled plotter modified to have a cutting blade. The channels of the device are 25 mils wide. The stencil was placed adhesive side down onto a 1/16" thick acrylic substrate having a 33 mil aperture aligned with the inlet aperture of the splitting device (see top of Figure 8). Four 55 mil outlet apertures were cut with the same machine in a second piece of self-adhesive laminating tape, which was placed onto the splitting device with the outlet apertures generally aligned with the ends of the forked channels. A fluid sample injected into the device was split into four approximately equal portions. Figures 17A and 17B are photomicrographs showing water flowing through such a splitting device, at two stages of operation.

Example 9

A microfluidic device capable of filtering a sample with a built-in valve was constructed. Stencils were constructed by cutting channels out of a self-adhesive laminating sheet tape (Avery Dennison, LS10P, 73603) using a computer-controlled plotter modified to have a cutting blade. A filter chamber 104 was formed in the stencil, along with an inlet channel 103 and an outlet channel 107 (see Figure 7). Beyond the filter chamber 104, the outlet channel 107 branched off into a chamber 105 and a channel 109. The stencil was placed adhesive side down onto a 1/16" thick acrylic substrate having a 33 mil diameter aperture aligned with the ends of channels 103 and 109 (apertures 100 and 101, respectively, in Figure 7). The filter chamber 104 was filled as in Example 6. Once dry, a solid piece of LS10P was used to cover the filter chamber 104. Colored water was injected into the device. The fluid passed through

the filter chamber 104 (see Figures 18A and 18B) into chamber 105 and selectively filled chamber 105 (see Figure 18C). Finally, once chamber 105 became filled, the fluid passed into channel 109 (see Figure 18D). The apertures 100 and 101 in the acrylic substrate 102 were placed over the tape stencil in such a way that the capillary forces at the aperture/tape interface were apparently greater than the forces in the channel 109. A device such as this one is useful to purify and concentrate samples.

Example 10

A microfluidic device was constructed using a tape stencil as both part of the device and as a mold for a silicone replicate. Referring to Figure 19A, a silicone master mold was constructed by using a vinyl sign cutter to create a stencil by cutting a dumbbell-shaped portion comprising channel 250 and apertures 251 from a 2 mil thick vinyl sheet. The stencil was placed on an acrylic substrate. A small acrylic box was constructed around the stencil to keep the silicone from running off the surface. RTV615A silicone rubber (90%) was mixed with RTV615B curing agent (10%) (General Electric Comp., Waterford, NY). The silicone was degassed, poured into the box, and cured overnight. The stencil 252 shown in Figure 19B was constructed using the same technique described in Example 7. The final device was constructed by placing the silicone replicate onto a 1/16" thick acrylic substrate having apertures drilled at the positions of the silicone wells. The tape stencil 252 was mounted to the opposite side of the acrylic substrate, adhesive side down. Inlet and outlet apertures were drilled in another acrylic block and a piece of double-sided tape, and were mounted to the opposite ends of the tape stencil. Fluid was injected through the device, and no leakage was observed. Figure 19C is a photomicrograph of the fluid passing through the silicone replicate. This method of mold creation is very advantageous, since hundreds of molds can be created on a single sheet of vinyl in a few minutes.

The present invention described and claimed herein is not to be limited in scope by the specific embodiments herein disclosed, since these embodiments are intended merely to illustrate several aspects of the invention. All equivalent embodiments are intended to be within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

The disclosures of all references cited herein are incorporated by reference in their entireties.

WHAT IS CLAIMED IS:

1. A microfluidic device comprising:

first and second substrates; and

at least one stencil disposed between the first and second substrates so as to define one or more sealed microstructures therebetween, wherein a stencil is adhered to at least one of the first and second substrates by an adhesive.

2. The microfluidic device of claim 1, comprising a plurality of stencils.

3. The microfluidic device of claim 1, wherein the first and second substrates are substantially planar.

4. The microfluidic device of claim 1, wherein the adhesive comprises an adhesive selected from the group consisting of rubber-based adhesives, acrylic-based adhesives and gum-based adhesives.

5. The microfluidic device of claim 1, wherein the stencil is self-adhesive.

6. The microfluidic device of claim 1, wherein the stencil comprises an adhesive tape.

7. The microfluidic device of claim 6, wherein the adhesive tape has adhesive on one side.

8. The microfluidic device of claim 6, wherein the adhesive tape has adhesive on both sides.

9. The microfluidic device of claim 6, wherein the adhesive tape is selected from the group consisting of pressure-sensitive tapes, temperature-activated tapes, chemically-activated tapes and optically-activated tapes.

10. The microfluidic device of claim 6, wherein the adhesive tape comprises a material selected from the group consisting of Mylar®, nylon and polyester.

11. The microfluidic device of claim 1, wherein the stencil and at least one of the first and second substrates are ultrasonically welded together.

12. The microfluidic device of claim 1, wherein the stencil comprises a material selected from the group consisting of polymers, papers, fabrics and foils.

13. The microfluidic device of claim 12, wherein the stencil comprises a polymer selected from the group consisting of Mylar®, polyesters, polyimides, vinyls, acrylics, polycarbonates, Teflon®, Kapton®, polyurethanes, polyethylenes, polypropylenes, polyvinylidene fluorides, polytetrafluoroethylenes, nylons, polyethersulfones, acetal copolymers polyesterimides, polysulfones, polyphenylsulfones, ABS, polyvinylidene fluorides, polyphenylene oxides, and derivatives thereof.

14. The microfluidic device of claim 12, wherein the stencil comprises a fluorinated polymer.

15. The microfluidic device of claim 1, wherein the stencil is elastomeric.
16. The microfluidic device of claim 15, wherein the stencil comprises an elastomeric material selected from the group consisting of rubber, viton and silicone.
17. The microfluidic device of claim 1, wherein the stencil is die-cut.
18. The microfluidic device of claim 1, wherein at least one of the first and second substrates comprises a material selected from the group consisting of Mylar®, FR-4, polyester, glass, acrylic, polycarbonate and fiberglass.
19. The microfluidic device of claim 1, further comprising a sealant coat on at least a portion of one or more of the stencil, the first substrate and the second substrate.
20. The microfluidic device of claim 19, wherein the sealant coat comprises a silicone material.
21. The microfluidic device of claim 19, wherein the sealant coat comprises one or more materials selected from the group consisting of Teflon®, Avatrel®, Liquin®, fluorocarbons, fluorothermoplastics, polyvinylidene fluorides, acrylics, waxes, epoxies, solders, polymers, paints, oils and varnishes.
22. The microfluidic device of claim 19, wherein the sealant coat is applied by spin-deposition.
23. The microfluidic device of claim 19, wherein the sealant coat is applied by spraying.
24. The microfluidic device of claim 19, wherein the sealant coat is applied by dipping.
25. The microfluidic device of claim 1, wherein the microstructure comprises a channel or a chamber.
26. The microfluidic device of claim 1, wherein the microstructure is at least partially filled with a filling material.
27. The microfluidic device of claim 26, wherein the filling material is a filter material.
28. The microfluidic device of claim 27, wherein the filter material is selected from the group consisting of polycarbonates, acrylics, acrylamides, polyurethanes, polyethylenes, polypropylenes, polyvinylidene fluorides, polytetrafluoroethylenes, naphion, nylons and polyethersulfones.
29. The microfluidic device of claim 27, wherein the filter material is selected from the group consisting of agarose, alginate, starch and carrageenan.
30. The microfluidic device of claim 27, wherein the filter material is silica gel.
31. The microfluidic device of claim 27, wherein the filter material is selected from the group consisting of Sephadex® and Sephacil®.

32. The microfluidic device of claim 27, wherein the filter material is hydroxyapatite.

33. The microfluidic device of claim 26, wherein the filling material is applied by silk screening.

34. The microfluidic device of claim 26, wherein the filling material is applied by lithography.

35. The microfluidic device of claim 26, wherein the filling material is applied by pick-and-place techniques.

36. The microfluidic device of claim 1, wherein at least one substrate has one or more apertures.

37. The microfluidic device of claim 1, further comprising one or more valves.

38. The microfluidic device of claim 1, wherein the microstructure comprises a forked channel.

39. The microfluidic device of claim 1, further comprising at least one electrode.

40. The microfluidic device of claim 39, wherein the electrode is for detecting or measuring an electrical property of a fluid.

41. The microfluidic device of claim 39, wherein the electrode is for promoting electrophoretic or electrokinetic flow of a fluid.

42. The microfluidic device of claim 1, wherein at least a portion of at least one of the first substrate and the second substrate is adapted to permit transmission of an optical signal.

43. The microfluidic device of claim 1, further comprising one or more additional substrates sealingly engaged thereto.

44. The microfluidic device of claim 43, wherein a substrate comprises a circuit board having on a surface thereof a microstructure.

45. A modular microfluidic device according to claim 1, adapted to connect to another microfluidic device.

46. A microfluidic system comprising a plurality of microfluidic devices according to claim 1, wherein at least two of the microfluidic devices are configured to enable fluid communication with each other.

47. The microfluidic system of claim 46, wherein two or more of the microfluidic devices are layered to form a three-dimensional microfluidic system.

48. A method for simultaneously producing a plurality of microfluidic devices, the method comprising the steps of:

providing a first substrate;

layering on the first substrate one or more panels, each comprising an array of stencils; and

layering on the one or more panels a second substrate so as to define a plurality of microstructures therebetween.

49. The method of claim 48, wherein at least one of the first and second substrates has one or more apertures.

50. The method of claim 49, wherein at least one of the panels is aligned with at least one of the first and second substrates so that the apertures are in fluid communication with the microstructures.

51. The method of claim 50, wherein the alignment is provided by peg-and-hole alignment.

52. The method of claim 48, wherein the layering is provided by a converter.

53. Microfluidic devices prepared according to the method of claim 48.

54. A mold prepared using at least a portion of the stencil of claim 1 as a form for defining the mold.

55. The mold of claim 54, comprising a silicone material.

56. A microfluidic device comprising a microstructure prepared using the mold of claim 54.

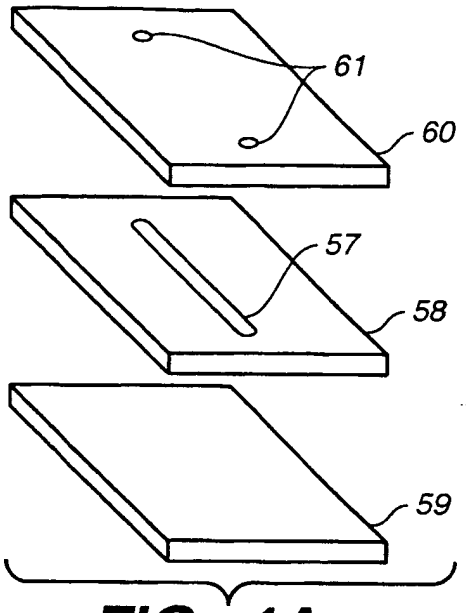


FIG._1A

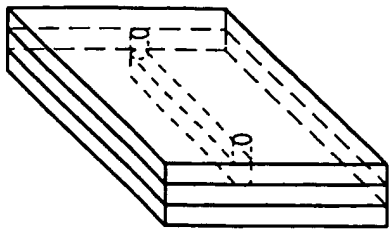


FIG._1B

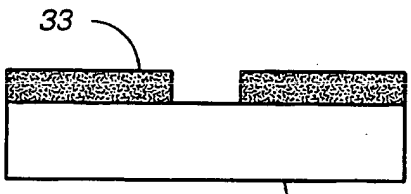


FIG._2A

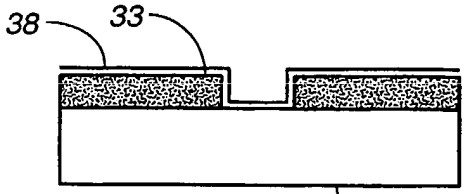


FIG._2B

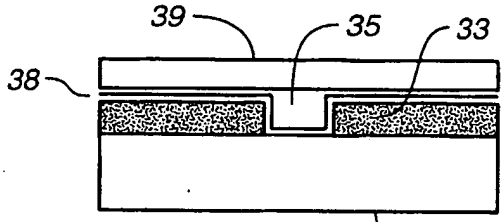


FIG._2C

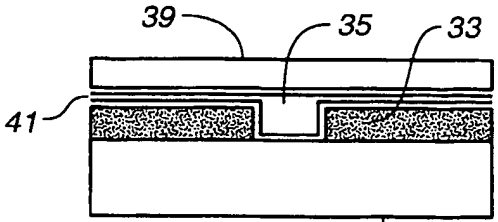


FIG._2D

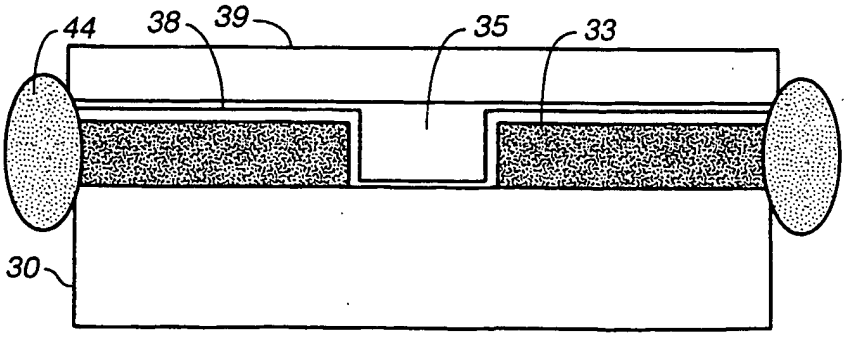


FIG._3

FIG._4A

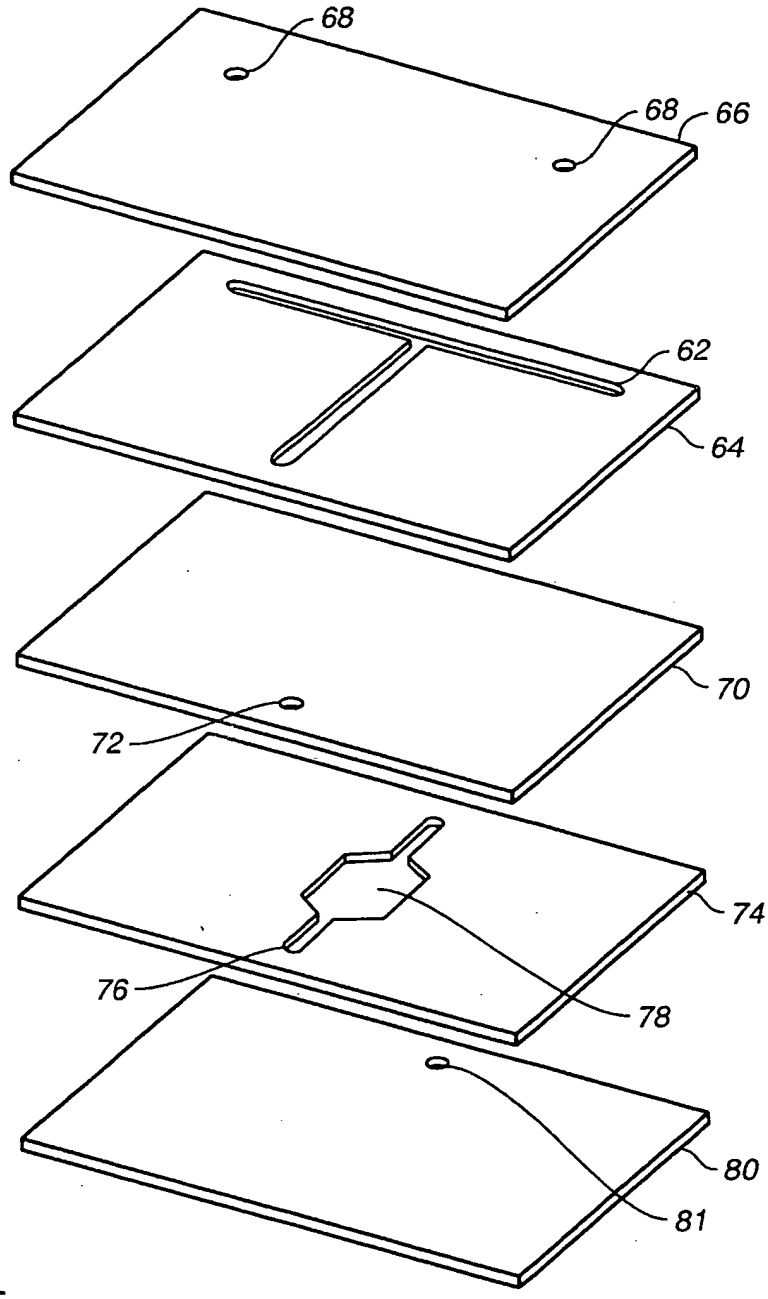


FIG. 4B

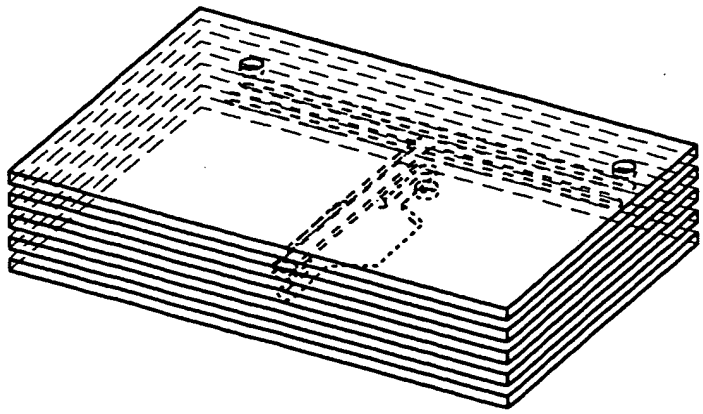


FIG._5A

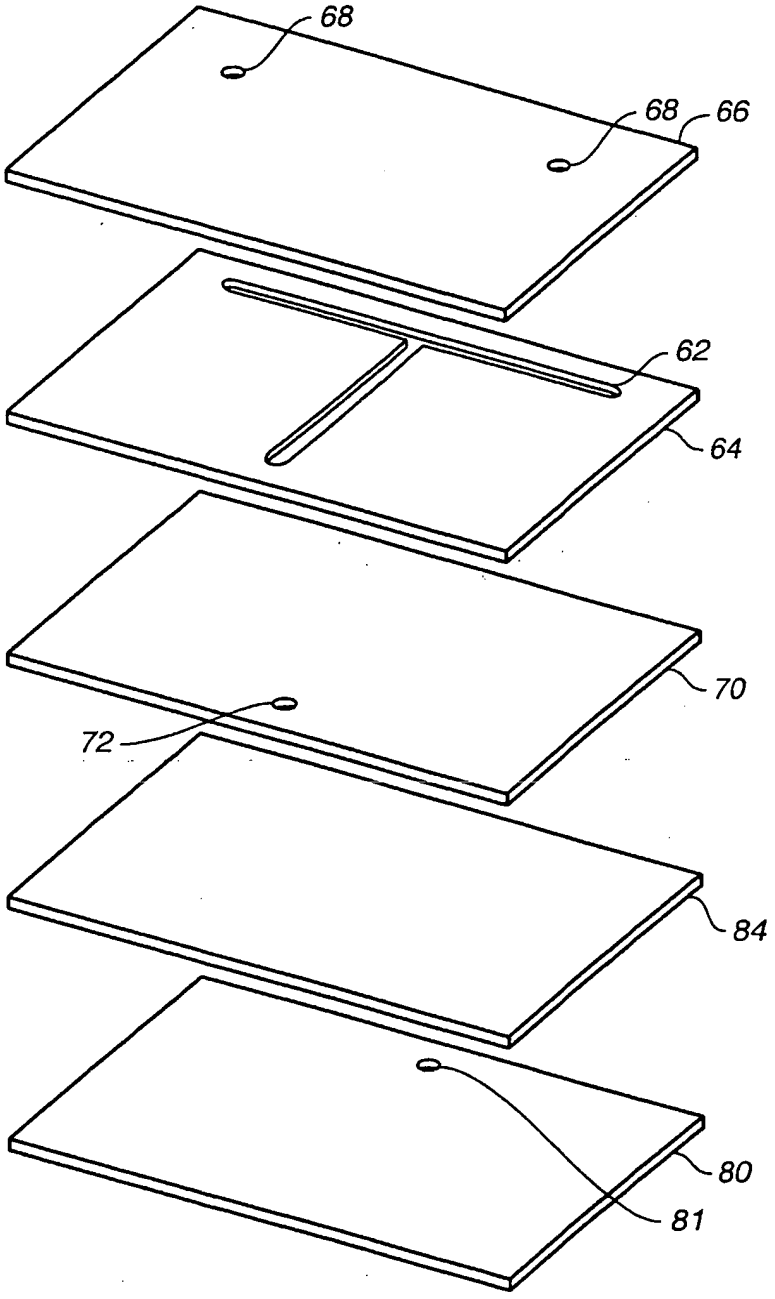
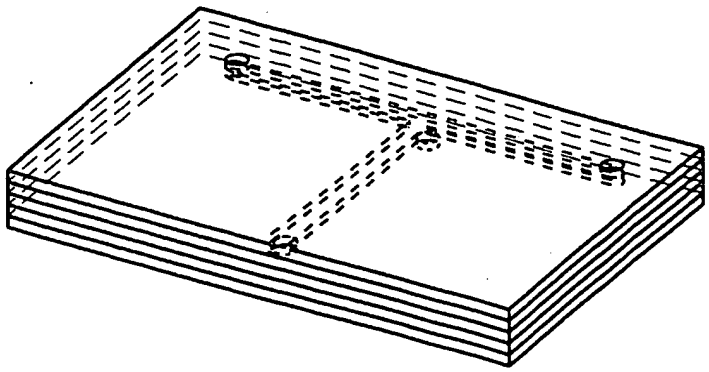


FIG._5B



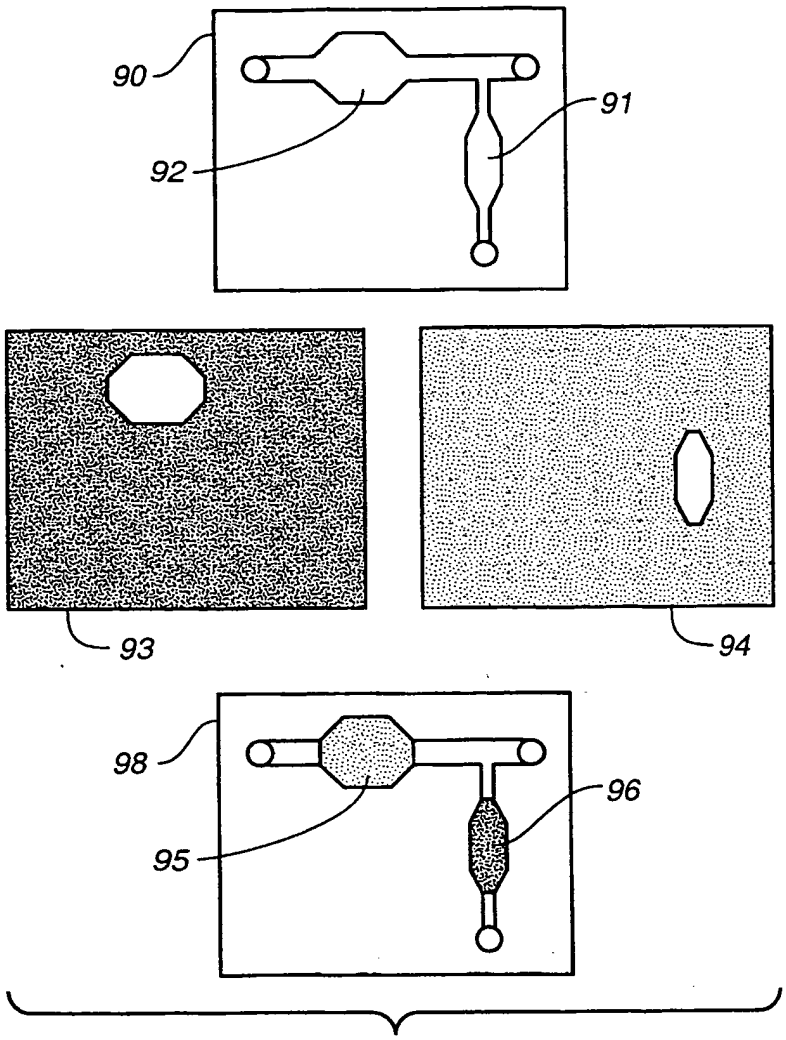


FIG._6A

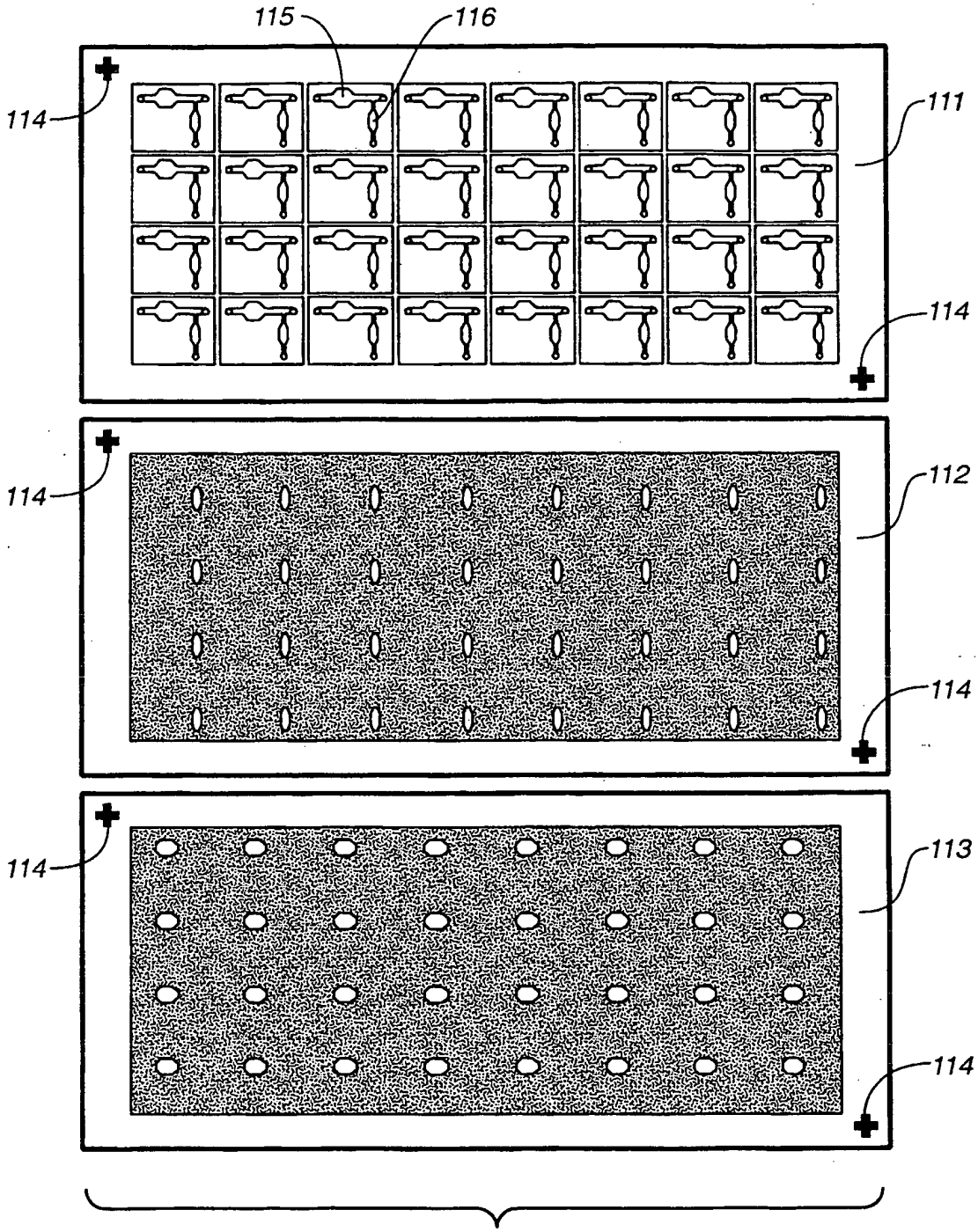
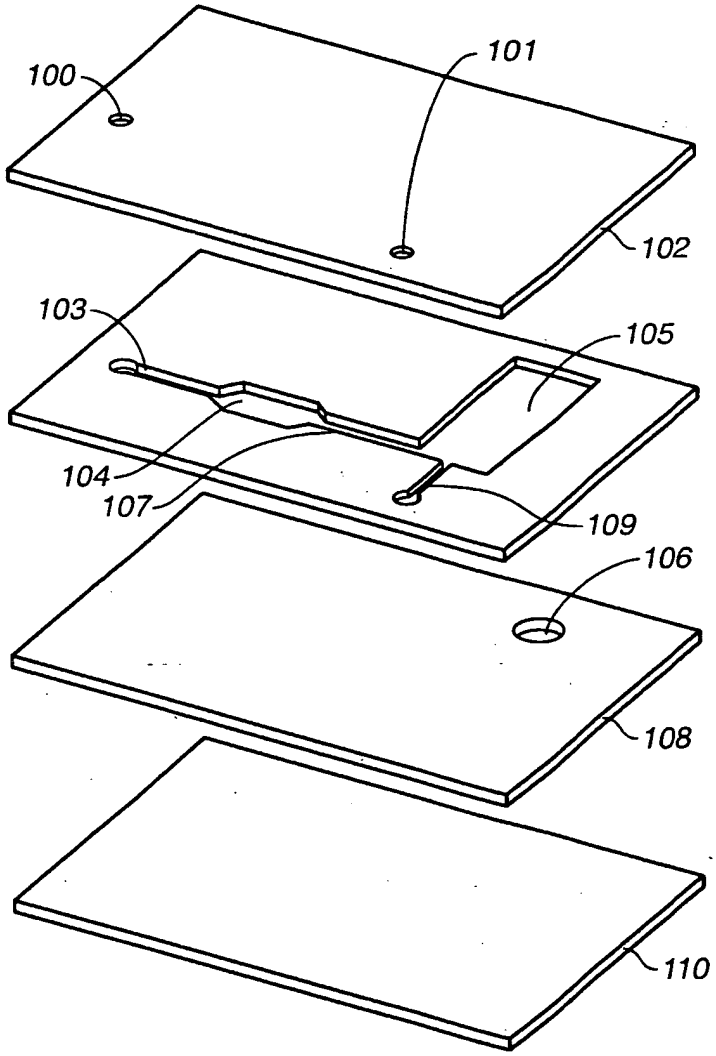


FIG._6B

FIG._7



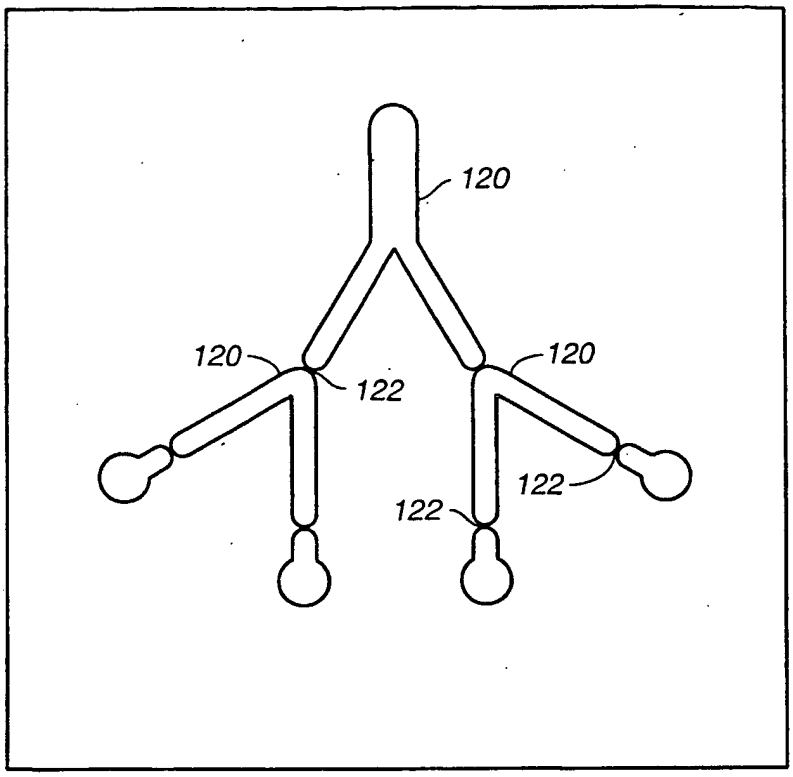


FIG._8

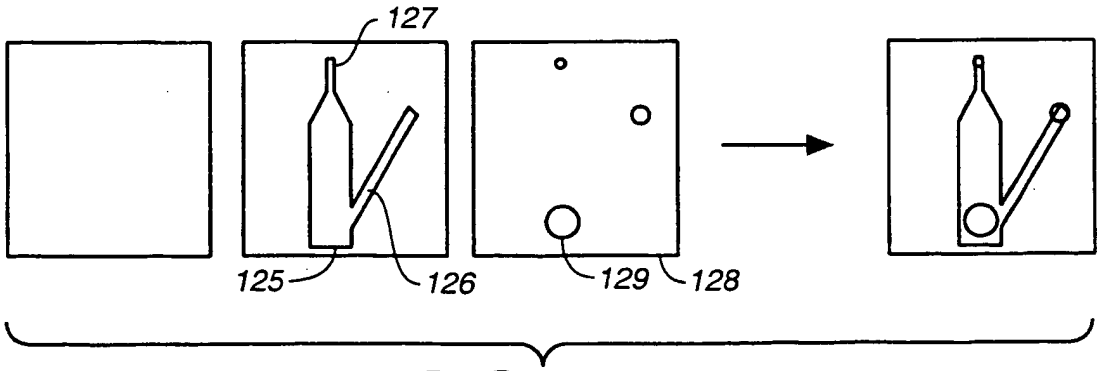


FIG._9

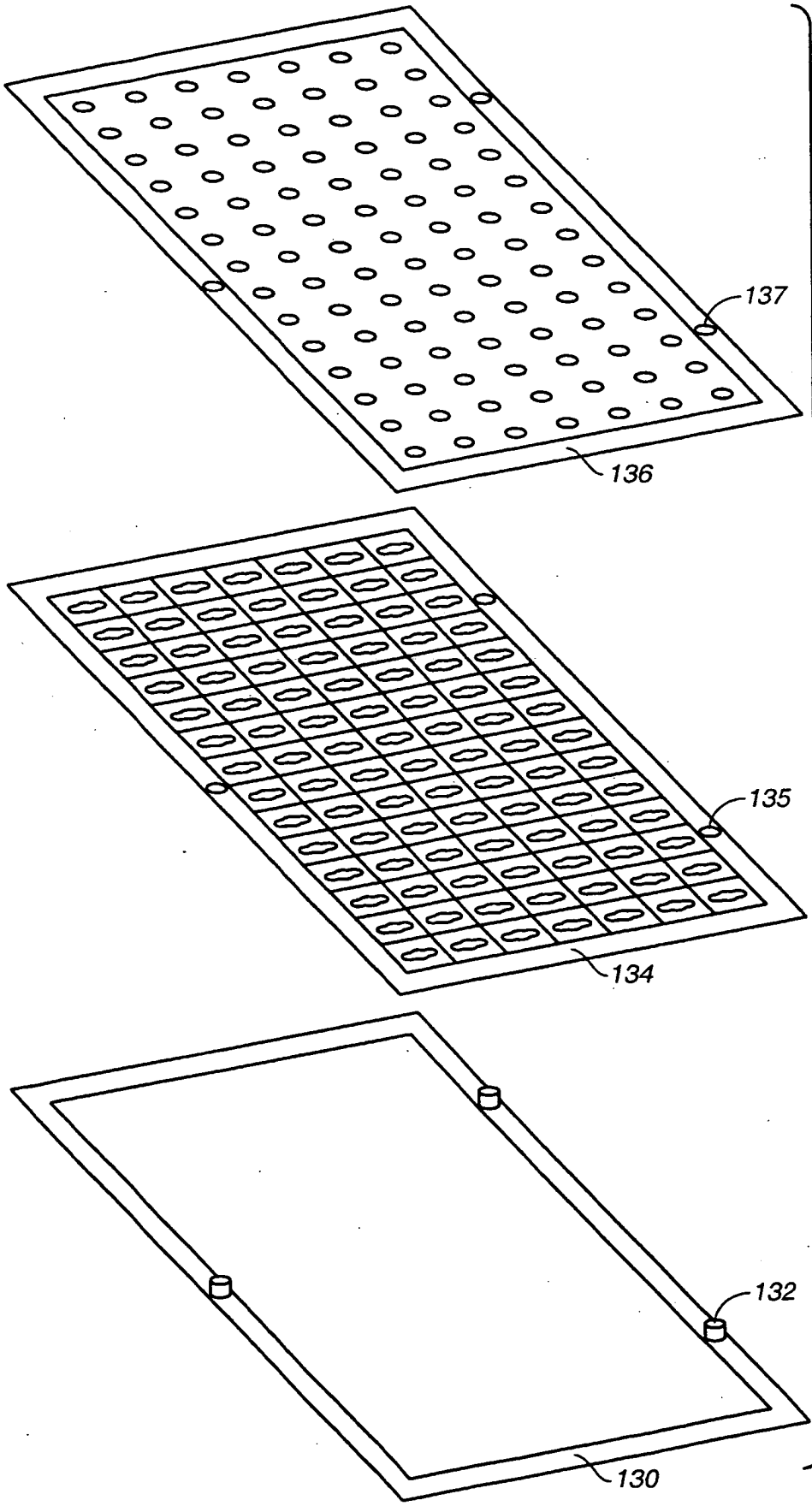


FIG._10

FIG._11A

FIG._11B

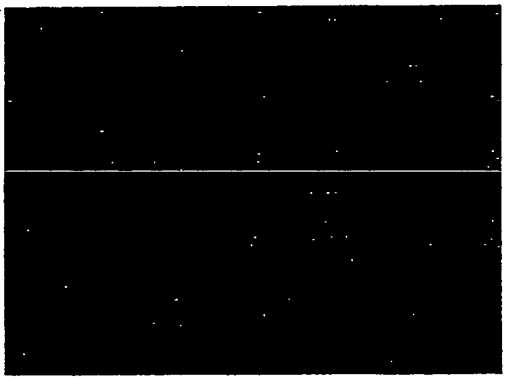


FIG._12B

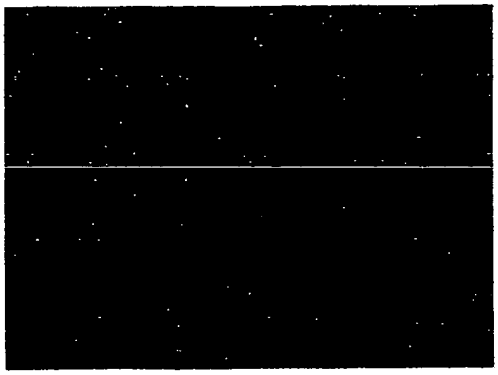


FIG._12C

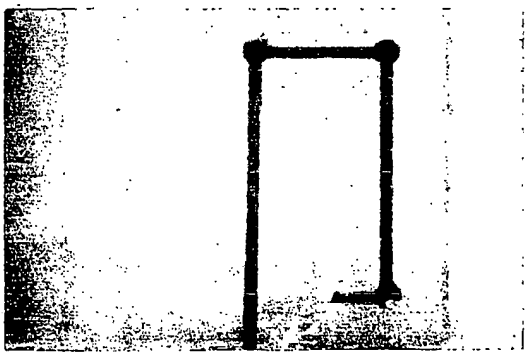


FIG._13B

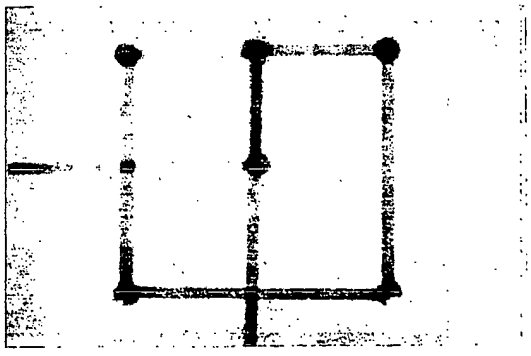


FIG._13C

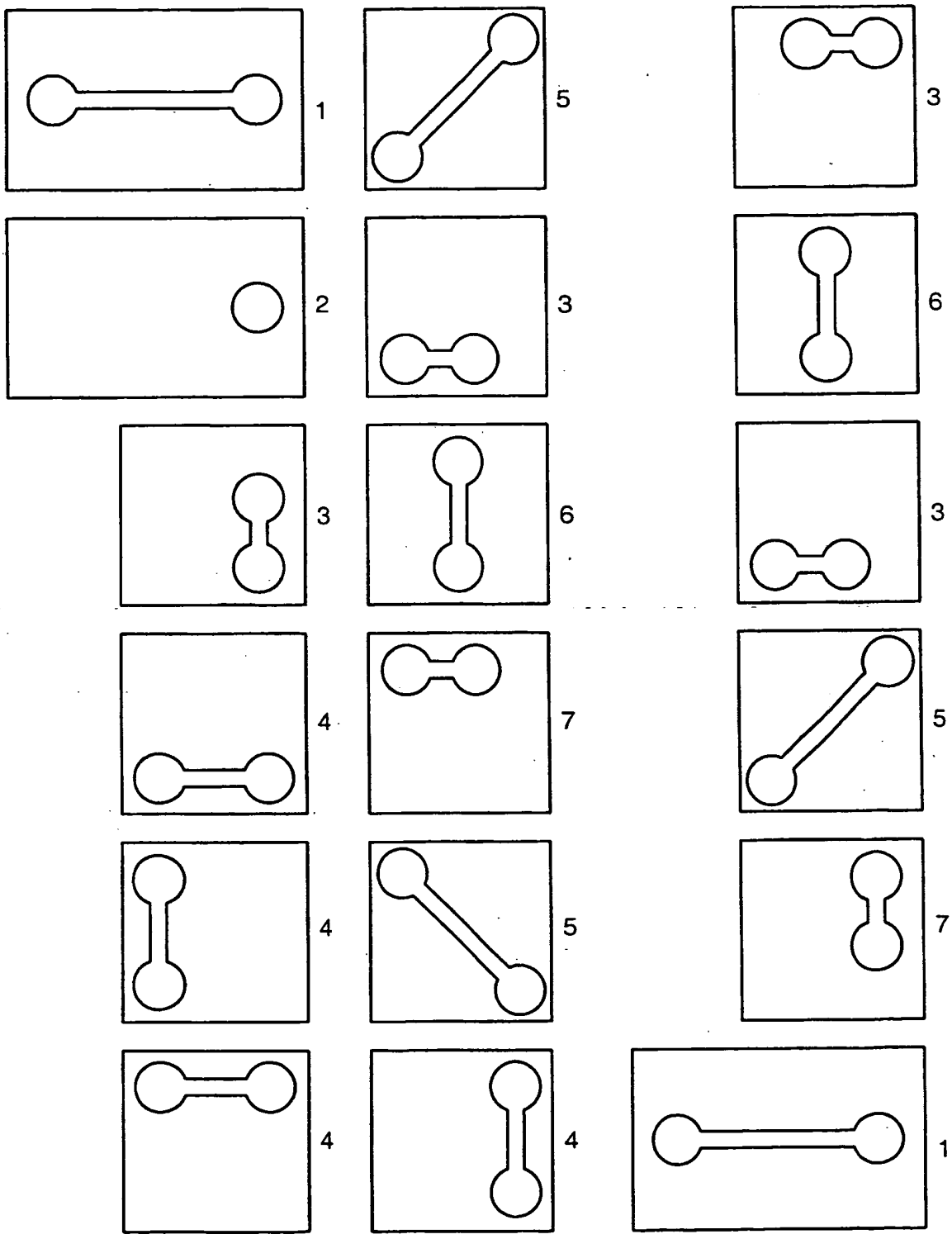


FIG._12A

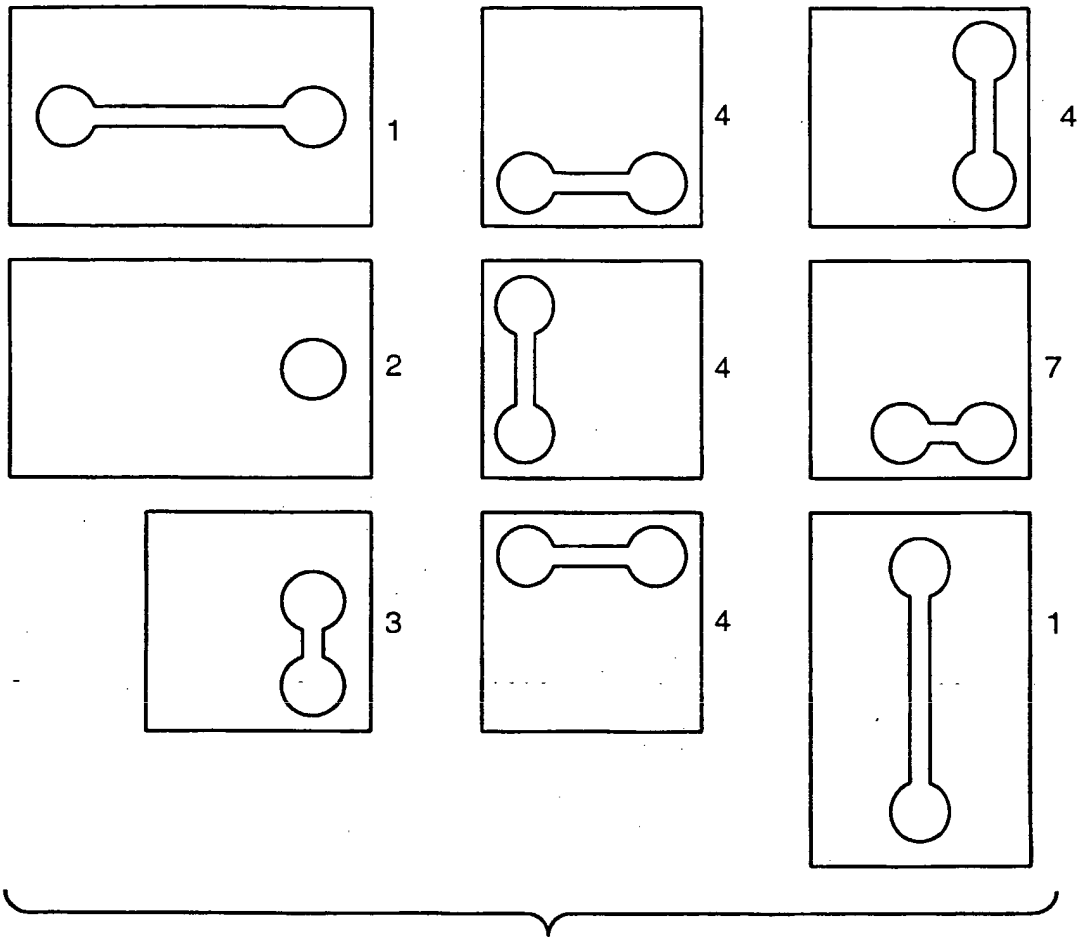


FIG._13A

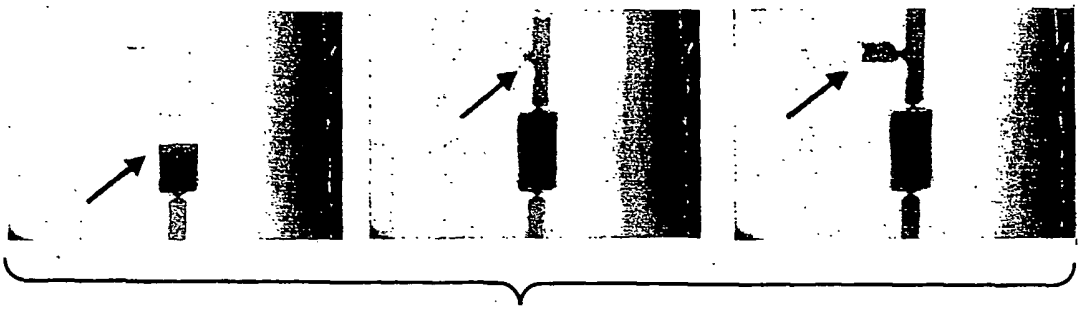


FIG._14

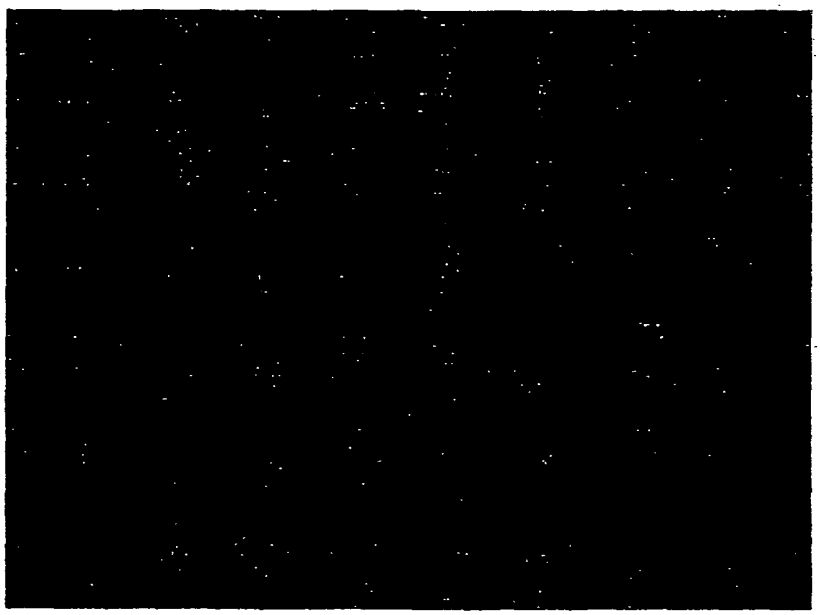


FIG._15

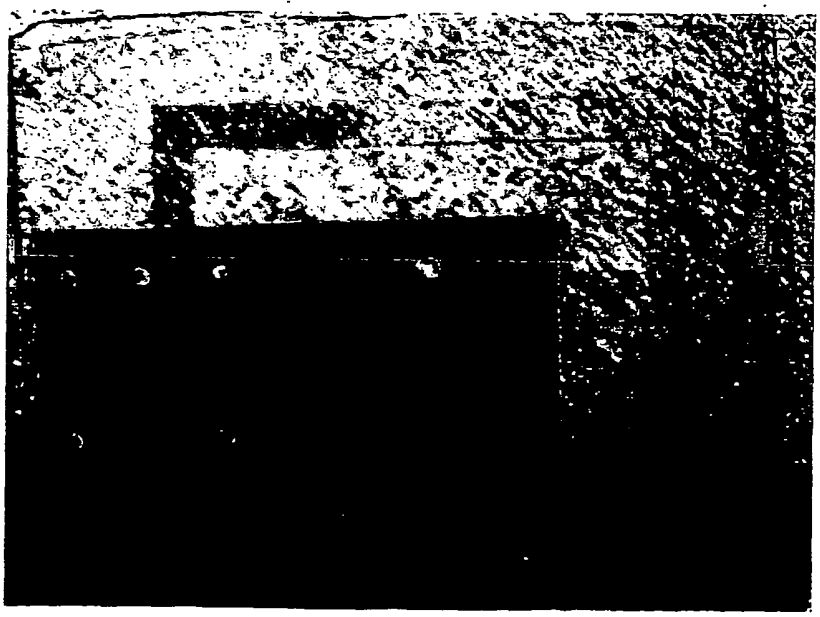


FIG._16B

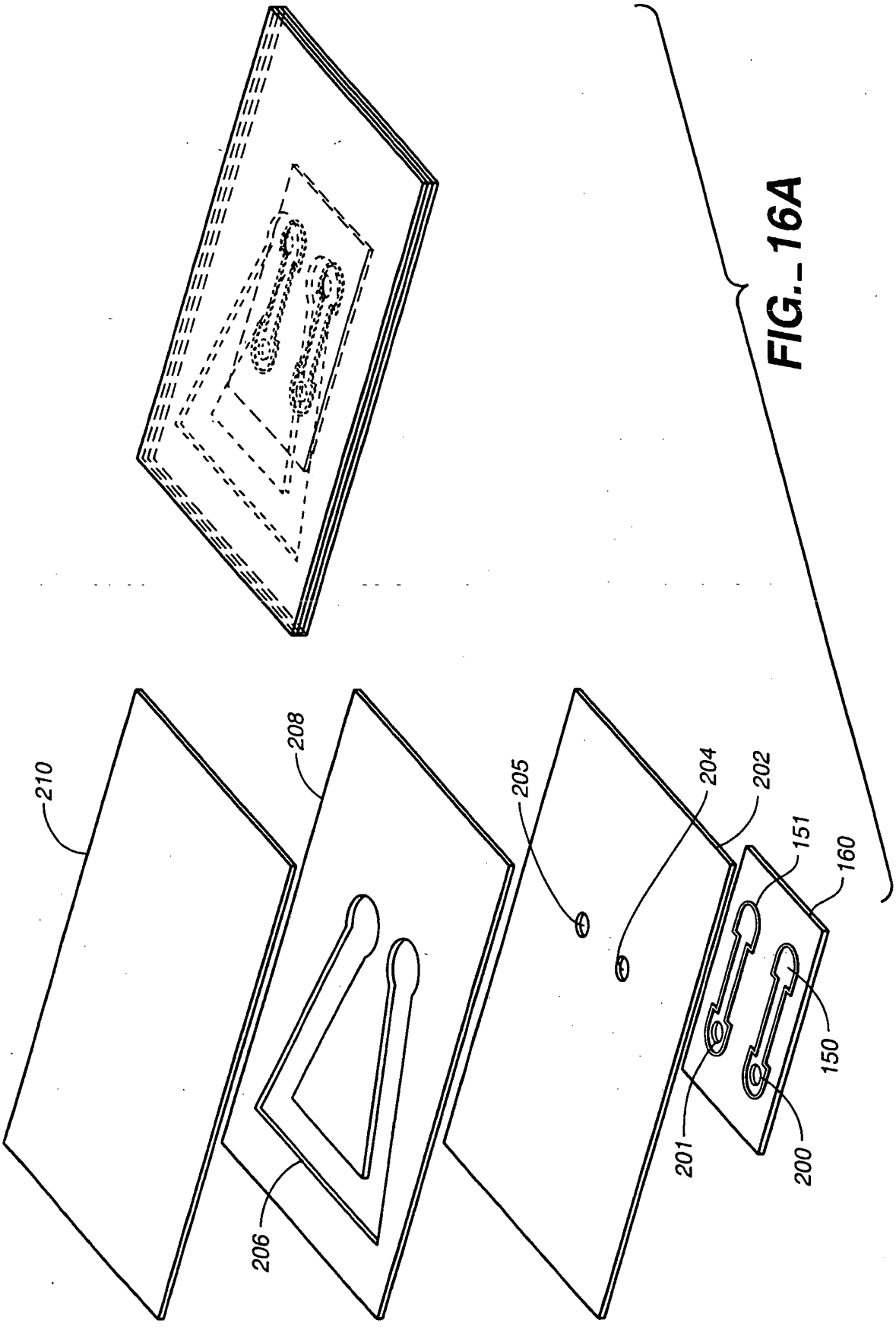


FIG._16A

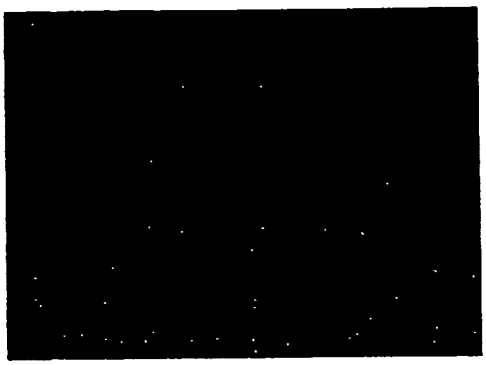


FIG._17A

FIG._17B

FIG._18A

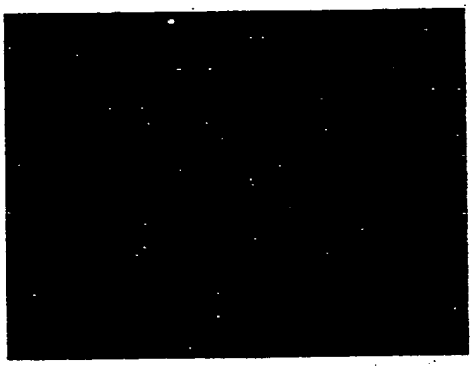


FIG._18B

FIG._18C

FIG._18D

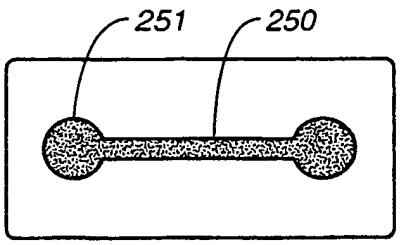


FIG._19A

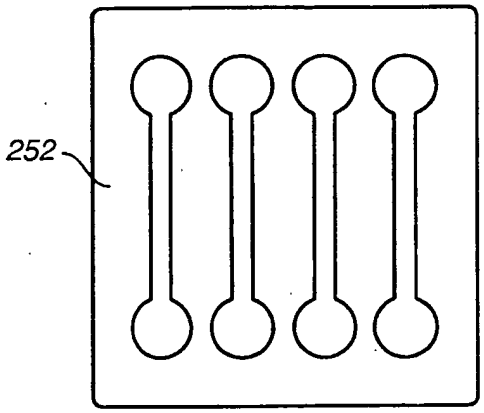


FIG._19B

FIG._19C

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/27366

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B81B1/00 G01N21/05 B01J19/00 B01L3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B81B G01N B01J B01L B81C F15B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 29497 A (CALIPER TECHN) 17 June 1999 (1999-06-17) page 2, line 28 -page 3, line 18 page 7, line 21 - line 26 page 25, paragraph 2 figures 5,6 --- -/--	1-10, 12-16, 18-21, 25-36, 38,43, 45-50, 52,53

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 February 2001	27/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	SLEIGHTHOLME, G

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 34909 A (SHAW) 15 July 1999 (1999-07-15) page 6, line 21 - line 23 page 8, line 30 - line 36 figures 13B,15D	1-4, 11-16, 18,36, 38, 43-51,53
X	CH 596 453 A (TSCHUDIN & HEID) 15 March 1978 (1978-03-15) column 1, line 66 -column 2, line 4; figures 1,2	1-10, 12-16, 18,25, 36,38, 43,46,47
X	FR 1 456 513 A (COMPAGNIE PARISIENNE D'OUTILLAGE) 11 January 1967 (1967-01-11) page 1, right-hand column, paragraph 4; figure 1	1,3,4, 12-16, 18,25, 36,38
X	FR 1 391 874 A (CONTINENTAL ELEKTROINDUSTRIE) 23 June 1965 (1965-06-23) page 1, right-hand column, paragraph 8 -page 2, left-hand column, paragraph 2	1,3,4, 12-16, 18,25, 36,38
A	WO 99 15888 A (ACLARA BIOSCIENCES) 1 April 1999 (1999-04-01) page 34, line 35 -page 35, line 21 page 36, line 31 - line 33 page 37, line 34 -page 38, line 8 page 45, line 20 -page 46, line 37 page 47, line 26 -page 48, line 10 figures 1-5	2,11-16, 18-24, 26-32, 34,35, 39-42, 48,54-56
A	MANZ A ET AL: "MICROMACHINING OF MONOCRYSTALLINE SILICON AND GLASS FOR CHEMICAL ANALYSIS SYSTEMS" TRAC, TRENDS IN ANALYTICAL CHEMISTRY,GB,ANALYTICAL CHEMISTRY. CAMBRIDGE, vol. 10, no. 5, 1 May 1991 (1991-05-01), pages 144-149, XP000201546 ISSN: 0165-9936 cited in the application abstract; figure 6	1,42,48

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DUFFY ET AL: "Rapid Prototyping of Microfluidic Systems in Poly(dimethylsiloxane)" ANALYTICAL CHEMISTRY,AMERICAN CHEMICAL SOCIETY. COLUMBUS,US, vol. 70, 1998, pages 4974-4984, XP002149044 ISSN: 0003-2700 cited in the application abstract; figure 1 ---	1,48,54
A	MARTYNOVA ET AL: "Fabrication of plastic microfluid channels by imprinting methods" ANALYTICAL CHEMISTRY, vol. 69, no. 23, 1 December 1997 (1997-12-01), pages 4783-4789, XP000955414 cited in the application abstract; figures 1,2 ---	1,48,54
A	MCCORMICK R M ET AL: "MICROCHANNEL ELECTROPHORETIC SEPARATIONS OF DNA IN INJECTION-MOLDEDPLASTIC SUBSTRATES" ANALYTICAL CHEMISTRY,US,AMERICAN CHEMICAL SOCIETY. COLUMBUS, vol. 69, no. 14, 15 July 1997 (1997-07-15), pages 2626-2630, XP000696569 ISSN: 0003-2700 cited in the application abstract ---	1,48,54
A	GONZALEZ C ET AL: "Fluidic interconnects for modular assembly of chemical microsystems" SENSORS AND ACTUATORS B,ELSEVIER SEQUOIA S.A., LAUSANNE,CH, vol. 49, no. 1-2, 25 June 1998 (1998-06-25), pages 40-45, XP004141435 ISSN: 0925-4005 cited in the application abstract ---	1,48
A	EP 0 846 875 A (XEROX CORP) 10 June 1998 (1998-06-10) abstract; figure 1 ---	2,18,19, 43-47
A	FR 2 582 359 A (SUEDDEUTSCHE KUEHLER BEHR) 28 November 1986 (1986-11-28) page 2, line 14 - line 33; figure 1 ---	5-7

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DE 197 39 722 A (FREDRICH) 1 April 1999 (1999-04-01) column 1, line 51 - line 64 column 2, line 27 - line 29 figures 2,4	18, 26-35, 43,44
A	US 5 858 194 A (BELL) 12 January 1999 (1999-01-12) column 6, line 49 -column 7, line 35; figures 7,9	26-35
A	WO 98 54568 A (PERSEPTIVE BIOSYSTEMS) 3 December 1998 (1998-12-03) page 5, paragraph 2; figure 1C	39-41

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No
PCT/US 00/27366

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